DGP 2023 GIESSEN

30th ANNUAL MEETING OF THE GERMAN SOCIETY FOR PARASITOLOGY

15–17 March 2023

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

ABSTRACTS

© JLU / Sebastian Ringleb

www.parasitology-meeting.de

TABLE OF CONTENTS

SCIENTIFIC PROGRAMME

Session I – Molecular Parasitology I • Protozoa 1 (*Plasmodium*)

A1

Understanding function and assembly of the atypical centrosome in *Plasmodium falciparum*

Y. Voß[1], C. Wenz[1], C. Simon[1], V. Stürmer[1], T. P. Romao[1], S. Klaus[1], M. Machado[1], A. Klemmer[1], M. Ganter[1], M. Brochet[2], J. Guizetti[1]

[1]Heidelberg University, Centre for Infectious Diseases, Heidelberg, Germany
[2]University of Geneva, Geneva, Switzerland

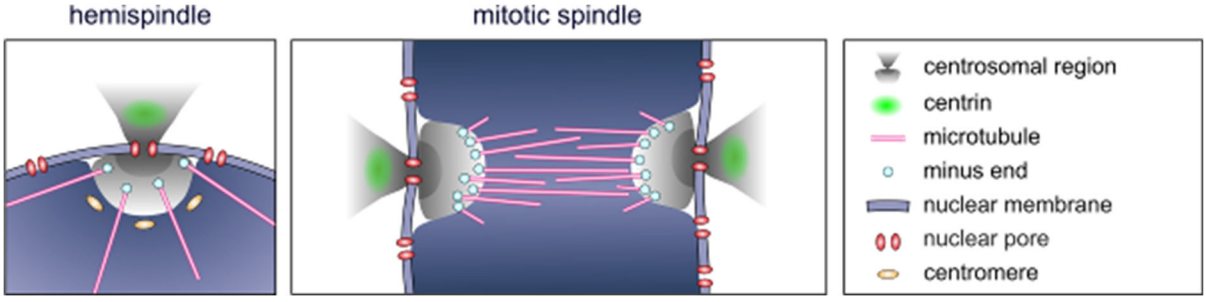
Throughout its life cycle Plasmodium falciparum undergoes several stages of extensive proliferation that are integral to survival and transmission of this malaria-causing parasite. Rapid parasite multiplication in the human red blood cell occurs via an atypical cell division mode, called schizogony, and relies on a particularly divergent centrosome. This so called centriolar plaque, functions as the main microtubule organizing center (MTOC) for the mitotic spindle and lacks conventional structures, such as centrioles. Our previous investigation using a combination of super-resolution, live cell, and correlative microscopy have revealed that it rather consists of two amorphous protein dense regions spanning across the nuclear membrane (see Figure). Composition and assembly of this atypical centrosome remain largely unknown.

To better understand the centriolar plaque we investigated centrins, which are small calcium-binding proteins and among the very few universally conserved MTOC components. Contrary to previous hypotheses we found liquid-liquid phase-separation (LLPS) as a driver behind the calcium-inducible self-assembly of some centrins in vitro. LLPS has emerged as biophysical model explaining the accumulation of concentrated proteins in droplet-like structures, which can contribute to the formation of membraneless compartments, such as centrosomes. To verify LLPS of PfCentrin1 in parasites we employed live cell STED and designed a novel inducible overexpression system.

Co-immunoprecipitation of PfCentrin1 further revealed a Sfi1-like protein (PfSlp) as novel centriolar plaque component. Conditional knock down of PfSlp caused a growth delay in blood stages and a reduced number of daughter cells. Surprisingly, intranuclear tubulin abundance was significantly increased, which raises the hypothesis that the centriolar plaque might be implicated in regulating tubulin homeostasis. Time-lapse microscopy revealed that this misregulation prevented or delayed spindle extension.

Taken together our data indicates that the centrin/Slp protein pair is a core component of the centriolar plaque and might implicate phase separation in its assembly.

Fig. 1



A2

Investigating the mechanism of heterogeneous protein accumulation among the nuclei of a *Plasmodium falciparum* schizont

A. Kudulyte[1], *S. Klaus*[1], *J. Niethammer*[1], *W. Straßburger*[1], *M. Ganter*[1]

[1]Center for Infectious Diseases, Heidelberg, Germany

Plasmodium falciparum proliferates through schizogony in the clinically relevant blood stage of infection. During schizogony, the nuclei multiply several times and the parasite develops into a multinucleated stage before cellularization occurs and daughter cells are released. To facilitate rapid parasite proliferation, the nuclei multiply asynchronously despite residing in the same cytoplasm. Moreover, the DNA replication fork protein *P. falciparum* PCNA1 accumulates only in those nuclei that replicate their DNA. However, the molecular mechanisms that enable heterogeneous nuclear accumulation of *P. falciparum* PCNA1 and thus asynchronous nuclear multiplication are not yet understood. To investigate this phenomenon, we used super-resolution live-cell microscopy. Our data suggest that *P. falciparum* PCNA1 initially accumulates close to the centrosome before relocating towards the nuclear periphery. This may indicate that the progression of the S-phase follows a conserved intranuclear pattern and that PCNA1 accumulation in subnuclear compartments underlies a specific spatiotemporal control. To elucidate this regulation, we are using mutagenesis and synthetic approaches to analyse how different protein motifs of *P. falciparum* PCNA1 contribute to its heterogeneous accumulation. Our data suggest that several amino acids play an important role. This work also identified a poorly conserved *P. falciparum* PCNA1 lysine-rich motif that was sufficient to accumulate GFP at the centrosomes. Building on these findings, we are now testing the behaviour of additional mutants and profiling the interactomes of wild-type and mutant *P. falciparum* PCNA1. Together, this will grant further molecular insight into the underlying mechanism of heterogeneous protein accumulation among *P. falciparum* nuclei.

A3

The histone methyltransferase PfSET2 regulates gene expression crucial for gametocyte development

S. Musa[1], J. P. Musabyimana[1], S. Wilch[1], J. Ngwa[1], G. Pradel[1]

[1]RWTH Aachen University, Division of Cellular and Applied Infection Biology, Aachen, Germany

The intracellular and extracellular survival of the malaria parasite *Plasmodium falciparum* in the human and mosquito hosts is dependent on rapid morphological and physiological changes, which are coordinated by various gene regulation mechanisms. Growing evidence suggests the contribution of epigenetic control mechanisms, in particular histone post-translational modifications, during intraerythrocytic replication and immune evasion of the asexual blood stage parasites. In contrast, the role of histone post-translational modifications during the sexual development of the parasite is not well studied. Previously conducted chemical loss-of-function studies by Ngwa et al. (2019), using the histone methyltransferase inhibitor BIX-01294, reveiled significant changes in the gene expression pattern of gametocytes and an impairment in gametocyte development and gametogenesis. In this study, we aimed to investigate the role of the histone methyltransferase PfSET2, one of ten known SET proteins of *P. falciparum*, in gene regulation during gametocyte development. We show that PfSET2 is expressed in the asexual and sexual blood stages. PfSET2 gene disruption results in changes in the histone methylation and gene expression patterns of the asexual blood stages and in the abrogation of gametocyte development. Our data point to a pivotal function of PfSET2 in the epigenetic regulation of gene expression crucial for gametocyte vitality.

A4

How a patatin-like phospholipase rules mitochondrial function in malaria parasites

E. Pietsch[1,2,3], A. Ramaprasad[4], S. Bielfeld[1,2,3], Y. Wohlfarter[5], B. Maco[6], L. Wilcke[1,2,3], J. Kloehn[6], M. A. Keller[5], D. Soldati-Favre[6], M. J. Blackman[4,7], T. W. Gilberger[1,2,3], P. C. Burda[1,2,3]

[1]Centre for Structural Systems Biology, Hamburg, Germany
[2]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[3]University of Hamburg, Hamburg, Germany
[4]The Francis Crick Institute, London, United Kingdom
[5]Medical University of Innsbruck, Innsbruck, Austria
[6]University of Geneva, Geneva, Switzerland
[7]London School of Hygiene & Tropical Medicine, London, United Kingdom

Introduction: *Plasmodium* parasites rely on a functional electron transport chain (ETC) within their mitochondrion for proliferation, and compounds targeting mitochondrial functions are validated antimalarials. In other organisms, mitochondrial functions have been shown to be heavily influenced by the phospholipid composition of mitochondrial membranes. However, the importance of phospholipid-modifying enzymes for mitochondrial function in *Plasmodium* parasites has not been analyzed so far.

Objectives: In this study, we aim to characterize the physiological function of the *Plasmodium falciparum* patatin-like phospholipase 2 (*Pf*PNPLA2, PF3D7_1358000) during asexual and sexual development of the parasite.

Materials & methods: We endogenously tagged *Pf*PNPLA2 with green fluorescent protein and generated *Pf*PNPLA2-deficient parasites using reverse genetics. Subsequently, we analyzed transgenic parasites using fluorescence microscopy and various cellular assays.

Results: We show that *Pf*PNPLA2 resides in the mitochondrion and its experimental deletion impairs asexual replication. The physiological consequences of this *Pf*PNPLA2 deletion are i) hypersensitivity to proguanil and inhibitors of the mitochondrial ETC including atovaquone, ii) lower mitochondrial respiration measured by quantification of oxygen consumption rates, and iii) a reduced mitochondrial membrane potential. Subsequent lipidomics-based analysis of the mitochondrial phospholipid cardiolipin (CL) reveals that deletion of *Pf*PNPLA2 is associated with an increase of CL with shorter and more saturated acyl-groups, suggesting a potential role of *Pf*PNLA2 in CL remodelling. We finally show that *Pf*PNPLA2-deficient parasites also display profound defects in gametocytogenesis with most parasites only developing to stage III gametocytes, underlining the importance of a functional mitochondrial ETC for sexual development of the parasite.

Conclusion: *Pf*PNPLA2 has a key role for mitochondrial function that is important for asexual replication and sexual development of the parasite.

A5

Expansion of a malarial parasite antigen family in the mammalian host can be explained by synthetic viability of a lethal *Plasmodium berghei* cysteine protease defect

E. Putrianti[1], *D. Fechtig*[2], *K. Matuschewski*[2], *K. Müller*[2]

[1]Max Planck Institute for Infection Biology, Parasitology Unit, Berlin, Germany
[2]Institute of Biology, Humboldt Universität zu Berlin, Molecular Parasitology , Berlin, Germany

During life cycle progression of malarial parasites, efficient and timely *Plasmodium* exit events from replication-permissive compartments are central steps in parasite stage conversion. Papain-like cysteine proteases of a family of *Plasmodium*-specific serine repeat antigens (SERAs) are central mediators of parasite egress and conserved across the genus *Plasmodium*. One member, termed SERA3 in the murine malaria model, is likely essential during blood infection. In this study, we employed a systematic reverse genetics approach to characterize the function of *P. berghei* SERA3. As predicted, we failed to generate *P. berghei* parasites lacking *SERA3* by targeted gene deletion. Surprisingly, in the presence of additional gene deletions, *i.e.* deletion of both active site serine (SERAser) members of the protease family, SERA1 and 2, parasites were viable and progressed through the parasite life cycle. Systematic phenotyping of *sera1-3(-)* parasites revealed that infected mice fail to develop signature symptoms of cerebral malaria and are able to clear an infection. Importantly, parasite clearance cannot be attributed to reduced virulence, since it only occurs when initiated by sporozoite infection, but not via transfusion of *sera1-3(-)* blood stages. Parasite clearance is strictly B- and T-cell dependent and elicits high titres of anti-parasite antibodies for up to one year. Intriguingly, the *sera1-3* triple knockout is a genocopy of the avian malarial parasite SERA repertoire. Apparently, SERA3 dysfunction is incompatible with asexual parasite growth in the presence of functional SERAser proteins. This suggests that the up to six SERAser protease family members could only evolve after fixation of SERA3 in the mammalian genomes. This genetic observation, known as synthetic viability, leads us to propose that expansion of the *SERA* gene family was vital to establish chronic and repeated *Plasmodium* infections in the mammalian host.

Session II – Veterinary Parasitology & Wildlife Parasites I

A6

Winter activity of *I. ricinus* and *D. reticulatus* in Germany – results from field studies and a veterinarian submission study

J. Probst[1], A. Springer[1], O. Kahl[2], M. Bröker[3], C. Strube[1]

[1]Institute for Parasitology, Centre for Infection Medicine, University of Veterinary Medicine Hannover, Hannover, Germany
[2]tick-radar GmbH, Berlin, Germany
[3]Global Health Press, Marburg, Germany

Introduction

The most important tick species in Germany are *Ixodes ricinus*, vector of the causative agents of Lyme borreliosis and granulocytic anaplasmosis, rarely found during colder months, and *Dermacentor reticulatus*, vector of the potentially fatal agent of canine babesiosis, known to be more cold tolerant.

Objectives

We provide an update on the seasonal activity pattern and possible winter activity of both species to assess whether current national recommendations on tick control in dogs and cats might need adaptations.

Materials & methods

Tick activity was monitored at nine collection sites via flagging (April 2020-April 2022), as well as in quasi-natural field plots. In a nationwide submission study (May 2020-June 2021), veterinarians sent in ticks from cats and dogs.

Results

An average of two *I. ricinus* and 23 *D. reticulatus* were flagged per 100 m² between December 2020 and February 2021. In the winter 2021/22, one *I. ricinus* and three *D. reticulatus* were collected on average. *Ixodes ricinus* winter activity in tick plots started in February 2021 (4.6%; 16/350), followed by a mean daily winter activity of 1.1% (4/350) in 2021/22. *Dermacentor reticulatus* showed a mean activity of 20% (20/100) in the winter 2020/21 and 14.7% (22/150) in 2021/22. Of the 19,514 submitted ticks, 211 *I. ricinus* and 324 *D. reticulatus* were collected in the winter 2020/21.

Conclusion

All study approaches demonstrated winter activity of both tick species. To protect dogs and cats with outdoor access from ticks and associated pathogens, an effective year-round tick control is strongly recommended.

A7

Cold plasma: a new option for poultry red mite control in laying hen farms?

V. Rüster[1,2], H. Werner[3], S. Wieneke[3], G. Avramidis[3], L. ten Bosch[3], C. Strube[1], T. Bartels[2]

[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hannover, Germany
[2]Friedrich-Loeffler-Institut, Institute of Animal Welfare and Animal Husbandry, Celle, Germany
[3]University of Applied Sciences and Art, Faculty of Engineering and Health, Göttingen, Germany

The poultry red mite (PRM), *Dermanyssus gallinae*, is one of the most common ectoparasites in poultry farming worldwide and can lead to substantial animal health and economic damages. The main problem in control is the rapid development of resistance to a wide range of chemical acaricides. Therefore, research on sustainable and effective control measures is becoming increasingly important.

In the present study, the acaricidal effects of cold atmospheric pressure plasma (CAPP) treatment on different development stages of the PRM were investigated under laboratory conditions. For this purpose, mites and mite eggs were exposed to a single plasma pulse generated under different plasma settings, using the ambient air gas mixture at atmospheric pressure.

The results showed that all developmental stages of the PRM could be killed by CAPP treatment. The mortality rates showed significant stage-specific differences and increased with progressing time after plasma exposure. The average mortality rate was 99.7% after 12 hours in all plasma-exposed mites.

The results show that CAPP has acaricidal effects on all developmental stages of the PRM, including eggs. Further studies are needed to show whether this method can also be used under practical conditions in occupied laying hen houses for efficient control of PRM infestations.

Acknowledgement: The project is supported by funds of the German Government´s Special Purpose Fund held at Landwirtschaftliche Rentenbank.

A8

Use of medicinal plants for helminth control – *in vitro* effect of a hydroethanolic extract from *Combretum mucronatum* leaves on larvae of gastrointestinal nematodes

F. N. Belga[1], M. K. Raulf[2], P. Waindok[2], V. Spiegler[3], A. Hensel[3], S. Rehbein[4], D. Ndjonka[1], C. Strube[2]

[1]University of Ngaoundéré, Faculty of Science, Department of Biological Sciences, Ngaoundéré, Cameroon
[2]University of Veterinary Medicine Hannover, Institute for Parasitology, Hannover, Germany
[3]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Münster, Germany
[4]Boehringer Ingelheim Vetmedica GmbH, Kathrinenhof Research Center, Rohrdorf, Germany

Combretum mucronatum is a forest liane that is used to treat parasite infections in parts of Western and Central Africa, where these infections are often diagnosed by traditional healers and herbal practitioners. However, anthelmintic properties of *C. mucronatum* on helminths of human and veterinary importance are insufficiently characterized.

To understand whether *C. mucronatum* leave extract (CME) can be a potential natural resource for the control of gastrointestinal nematodes, its activity against infectious larvae of parasitic nematodes was tested *in vitro*.

To this end, the inhibitory activity of hydroethanolic components of *C. mucronatum* leaves was evaluated by larval migration assays.

Even low concentrations of the CME had an inhibitory effect on the migration of larvae of *Ascaris suum* (IC50=5.5 µg/ml), *Ancylostoma caninum* (IC_{50}=19.0 µg/ml) and *Trichuris suis* (IC_{50}=7.4 µg/ml), whereas inhibition of larvae of *Toxocara canis* (IC_{50}=310.0 µg/ml) and *Toxocara cati* (IC_{50}=7249.8 µg/ml) was seen at higher doses only. While larvae of *Ostertagia ostertagi* (IC_{50}=48.9 µg/ml), *Cooperia oncophora* (IC_{50}=28.3 µg/ml) and *Trichostrongylus colubriformis* (IC_{50}=2.1 µg/ml) were highly sensitive to the plant extract, larvae of most other gastrointestinal strongylids were only moderately or slightly affected.

The larval migration assays indicated a high activity of CME against larvae of helminths of genera which contain also classical soil-transmitted nematodes of human health concern. Ongoing qPCR-based assays on the transcription of chemo resistance-inducing and detoxification-related genes may unravel mechanisms underlying this activity. Moreover, *in vivo* experiments with *H. contortus*-infected goats treated with different dosages of CME will show whether the extract is suitable for the treatment of *H. contortus* infections.

A9

Investigating the nemabiome using the COI marker region to decipher the cyathostomin diversity in equids

I. Diekmann[1], J. Krücken[1], S. Andreotti[1], S. Mbedi[2], T. Kuzmina[3], C. Bredtmann[1], H. Hofer[4], G. Sallé[5], F. Borges[6], M. de Freitas[6], J. Matthews[7], T. Tzelos[7], L. de Carvalho[8], M. Nielsen[9], S. Sparmann[2,10], G. von Samson-Himmelstjerna[1]

[1]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany
[2]Berlin Center for Genomics in Biodiversity Research, Berlin, Germany
[3]National Academy of Sciences of Ukraine, Kyiv, Ukraine
[4]Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
[5]INRAE - Université de Tours, Nouzilly, Germany
[6]Federal University of Mato Grosso do Sul, Campo Grande, Brazil
[7]Moredun Research Institute, Penicuik, United Kingdom
[8]University of Lisbon, Lisboa, Portugal
[9]University of Kentucky, Lexington, KY, United States
[10]Leibniz-Institut for Freshwater Ecology and Inland Fisheries, Berlin, Germany

Introduction

Cyathostomins are ubiquitous in equids. While the nematode genera of equids have in general low species diversity, the Cyathostominae with 51 accepted species are the exception and represent a highly diverse multi-species complex. They are the most common parasites in the large intestine of equids and can cause severe clinical symptoms due to simultaneous excystation of the encysted larvae into the gut lumen. Anthelmintic resistance in parasitic nematodes is widespread and, therefore, of considerable veterinary and economic importance. Morphological identification and differentiation of these species requires detailed knowledge of their morphology and intensive training.

Objective

Improved tools for species identification are needed to investigate the interaction and influence of competing species within an ecological niche in horse intestine. The cytochrome c oxidase I (CO1) gene represents an efficient marker to estimate strongyle species richness. Compared to the internal transcribed spacer 2 (ITS-2) marker region, this marker region allows a reliable differentiation between *Cylicostephanus calicatus* and *Coronocyclus coronatus.*

Materials and methods

Therefore, the CO1 marker region was used to perform the Next Generation Sequencing (NGS) approach to detect strongyle population diversity. To compare the nemabiome between hosts with different geographical background and different treatment histories, an average of 10 treated and 10 untreated horses from Brazil, Ukraine, Kentucky, Scotland and Germany were examined and strongyle third stage larvae (L3) were obtained. Using Illumina MiSeq v300 amplicons were sequenced and a bioinformatic data processing pipeline was developed which provides a tool to distinguish between the different cyathostomin species.

Results

A total of 144 samples were analysed. On average, 38,500 reads could be generated per sample. Using the metabarcoding-dual-indexing approach, as well as bioinformatic pipelines, it was possible to assign the non-overlapping sequences to each individual sample and Strongylidae species could be detected.

Conclusions

Metabarcoding is a powerful tool which enables molecular characterization and investigation of the diversity and dynamics of strongyle populations within and between different hosts. The evaluation of the NGS data set allows identifying of the different cyathostomin species, per animal and the effects of geographic regions and management practices.

A10

Comparison of different diagnostic methods for the detection of *Toxocara* spp. in faecal samples of cats and dogs

D. T. Winterfeld[1], M. Globokar[2], N. Pantchev[2], B. Schauer[3], S. Mouchantat[3], F. J. Conraths[1], J. Schulz[1], G. Schares[1], P. Maksimov[1]

[1]Friedrich-Loeffler-Institut, Institut für Epidemiologie, Greifswald, Germany
[2]IDEXX Laboratories, Kornwestheim, Germany
[3]Universitätsmedizin Greifswald, Institut für Community Medicine, Abteilung SHIP-KEF, Greifswald, Germany

Introduction:

Toxocara canis and *Toxocara cati* are parasitic nematodes that occur worldwide. Embryonated *Toxocara* spp. eggs in the environment pose a zoonotic risk, especially for children. Little is known about the diagnostic performance and comparison of different detection methods of *Toxocara* spp. in faeces of cats and dogs.

Objectives:

The aim of this study is to assess the analytical sensitivity of different methods for the detection of *Toxocara* spp. in faeces of cats and dogs. In addition, a high-throughput method has been developed to facilitate epidemiological studies at the species level.

Materials & methods:

Two automated DNA extraction methods using mechanical lysis and the King Fisher® flex system were compared regarding their performances. DNA was assessed for parasite-specific genome sequences by TaqMan® real-time PCR. All faecal samples were also examined using the sedimentation-flotation method to allow comparisons between molecular and conventional parasitological methods. Furthermore, a sieving method for the detection, enrichment and purification of parasite eggs in faecal samples was developed and subsequently applied.

Results:

The analytical sensitivity of real-time PCR in combination with automated DNA extraction methods was higher if King Fisher® in 96-well-plates was applied (limit of detection: < 1 egg/extraction [200 µl] (*T. cati*), 3 eggs/extraction [200 µl] (*T. canis*)) as compared to the use of 24-deep-well-plates (limit of detection: 2 eggs/extraction [1 ml] (*T. cati*), 28 eggs/extraction [1 ml] (*T. canis*)). The frequently used sedimentation-flotation method had a slightly lower analytical sensitivity (limit of detection: 78 eggs/sample) compared to the new established sieving method (limit of detection: 10 eggs/sample).

Conclusion:

Real-time PCR in combination with the automated DNA extraction in 96-well-plates and the sieving method had comparable analytical sensitivities. The sieving method was developed to purify and enrich parasite eggs, with a higher detection sensitivity than the sedimentation-flotation method. However, the method based on the automated DNA extraction and subsequent real-time PCR provides a significantly higher sample throughput. Thus, it allows the examination of at least five times more samples at the same time at species level. Therefore, this method is markedly more advantageous than the conventional flotation method from a labour, economic and epidemiological point of view.

A11

Typing of European *Toxoplasma gondii* type II strains by a novel Ion AmpliSeq-based method

M. Joeres[1], P. Maksimov[1], D. Höper[2], S. Calvelage[2], M. Fernández-Escobar[3], R. Calero-Bernal[3], B. Koudela[4], R. Blaga[5], M. Globokar Vrhovec[6], K. Stollberg[7], N. Bier[7], S. Sotiraki[8], J. Sroka[9], W. Piotrowska[9], P. Kodym[10], W. Basso[11], F. J. Conraths[1], A. Mercier[12], L. Galal[12], M. L. Dardé[12], A. Balea[13], F. Spano[14], S. Caccio[14], L. M. Ortega-Mora[3], P. Jokelainen[15], G. Schares[1]

[1]Friedrich-Loeffler-Institut, Institute of Epidemiology, HGW-Insel Riems, Germany
[2]Friedrich-Loeffler-Institut, Institute of Diagnostic Virology, HGW-Insel Riems, Germany
[3]Complutense University of Madrid, SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Madrid, Spain
[4]University of Veterinary Sciences, Central European Institute of Technology (CEITEC), Brno, Czech Republic
[5]ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, France
[6]IDEXX Laboratories, Kornwestheim, Germany
[7]German Federal Institute for Risk Assessment, Department for Biological Safety, Berlin, Germany
[8]Veterinary Research Institute, Hellenic Agricultural Organisation-Demeter, Thessaloniki, Greece
[9]National Veterinary Research Institute, Department of Parasitology and Invasive Diseases, Pulawy, Poland
[10]National Institute of Public Health, Centre of Epidemiology and Immunology, Prague, Czech Republic
[11]University of Bern, Institute of Parasitology, Vetsuisse Faculty, Bern, Switzerland
[12]EpiMaCT - Epidemiology of chronic diseases in tropical zone, Institute of Epidemiology and Tropical Neurology, Limoges, France
[13]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Department of Parasitology and Parasitic Diseases, Cluj-Napoca, Romania
[14]Italian National Institute of Health, Rome, Italy
[15]Statens Serum Institut, Infectious Disease Preparedness, Copenhagen, Denmark

Introduction

Toxoplasma gondii has a clonal population structure in Europe, where the type II clonal lineage prevails. A genotyping method based on 15 microsatellite (MS) regions represents the current reference standard.

Objectives

We aimed to establish a highly discriminatory next-generation sequencing (NGS)-based typing method for *T. gondii* to differentiate closely-related type II strains, to trace back infection sources and monitor for recombinant and emerging *T. gondii* strains.

Materials & methods

Toxoplasma gondii field isolates (n=110) were collected from different parts of Europe and assessed by whole genome sequencing (WGS). In comparison to ME49 (a type II reference), different highly polymorphic regions (HPRs) were identified, showing a considerable number of single nucleotide polymorphisms (SNPs). After confirmation by Sanger sequencing, 18 HPRs were used to design a primer panel for multiplex PCR in order to establish a multilocus Ion AmpliSeq-based typing method. The isolates analysed by WGS were typed with the novel method, and the sensitivity of the method was tested with serially diluted reference DNA samples.

Results

Type I, II and III clonal lineages were clearly separated by the 18 HPR Ion AmpliSeq typing technique. Two recombinant strains were correctly identified as type II x III strains. Among the type II strains, the method could differentiate a larger number of haplotypes compared to MS typing. The numbers and identities of SNPs were identical in outbreak isolates, indicating a common source. Furthermore, almost all SNPs identified by the method corresponded to those that were expected based on WGS. By testing serially diluted DNA samples, the technique exhibited similar analytical sensitivity as MS typing.

Conclusion

The results of our study show that reliable typing of *T. gondii* strains using an NGS-based multilocus typing method is feasible. The developed 18 HPR-based Ion AmpliSeq method has a high typing resolution, which appears promising for tracing infection sources in outbreaks and for detecting recombinant strains.

Funding: This work was done as part of TOXOSOURCES project, supported by funding from the European Union"s Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

Session III – Drug Development/Resistance

A12

Elucidation of Dihydroquinine Mechanism(s) of Action against *T. gondii*

D. A. Abugri[1], J. A. Ayariga[1], M. Boersma[2], A. Napier[1], B. K. Robertson[1]

[1]Alabama State University, Biological Sciences, Montgomery, AL, United States
[2]Auburn University, Auburn, AL, United States

Daniel A. Abugri1, 2, 3*, Joseph A. Ayariga1, Melissa Boersma4, Audrey Napier1, Boakai K Robertson1,2

1Department of Biological Sciences, 2Microbiology PhD Program, 3Laboratory of Ethnomedicine, Parasitology, and Drug Discovery, College of Science, Technology, Engineering and Mathematics, Alabama State University, Montgomery, AL, 36104, 4Department of Chemistry and Biochemistry, Auburn University, Auburn, AL, 36849

***** Corresponding Author**:** Daniel A. Abugri, Ph.D.

Email address: dabugri@alasu.edu;Tel:334-604-8158

Abstract

Dihydroquinine (DHQ) also known as hydroquinine, is a quinine-derived compound. DHQ has a history of inhibiting *Plasmodium falciparum*, and *Plasmodium berghei*, and possesses anti-arrhythmia properties. Though, previous studies in *Plasmodium* spp have shown its target to be nucleic acids and protein synthesis. Our team recently showed that it disrupts mitochondria membrane potential, upregulates ROS production, and depletes ATP production in *T. gondii*. To unify these observations with previously identified targets, we tested for the first time the effect of DHQ on *T. gondii* tachyzoites metabolites and lipid production in a concentration dependent manner. Interestingly, the multi-omics (metabolomics and lipidomics) studies showed that DHQ down-regulates certain lipid classes, nucleic acid precursors, and amino acid synthesis in a concentration-dependent manner. Also, for the first time, *in silico* analysis showed that DHQ binds strongly to DNA gyrase, Calcium Dependent Protein Kinase 1 (CDPK 1), and prolyl tRNA synthetase and thus could affect DNA replication and translational activities in *T. gondii*. In summary, our findings indicate that DHQ will be an effective anti-*T. gondii* agent and could be further developed for clinical use.

***Keywords**:* Dihydroquinine, metabolomics, lipidomic, DNA Gyrase, tRNA Synthetase, Calcium Dependent Protein Kinases, *T. gondii*

A13

In-vivo anticoccidial efficacy of green synthesized iron-oxide nanoparticles using leaf extract of *Ficus racemosa* Linn. (Moraceae) against *Emeria tenella* infection in broiler chickens

M. Afzal[1,2], *A. Khan*[2], *N. Qureshi*[2]

[1]University of Sialkot, Zoology, Sialkot, Pakistan
[2]Quaid-i-Azam University, Zoology, Islamabad, Pakistan

Introduction

Green synthesis of iron oxide nanoparticles (IONPs) using botanical extracts has recently fascinated substantial attention due to their environmental protection advantages, their low-cost, and their easy fabrication with the targeted applications.

Objectives & Methods

In the present study, IONPs were synthesized using the aqueous leaf extract of *Ficus racemosa* as a reducing and capping agent. These IONPs were evaluated for their impacts on the growth performance, biochemistry, blood profile, and histology in the coccidiodized broiler chicken with *Emeria tenella* under in vivo conditions. The confirmation of characteristics features of green synthesized IONPs was first obtained using UV-Vis spectroscopy, Fourier transforms infrared (FTIR), X-Ray diffraction (XRD), energy dispersive X-ray absorption (EDX), scanning electron microscopy (SEM), zeta potential and zeta size.

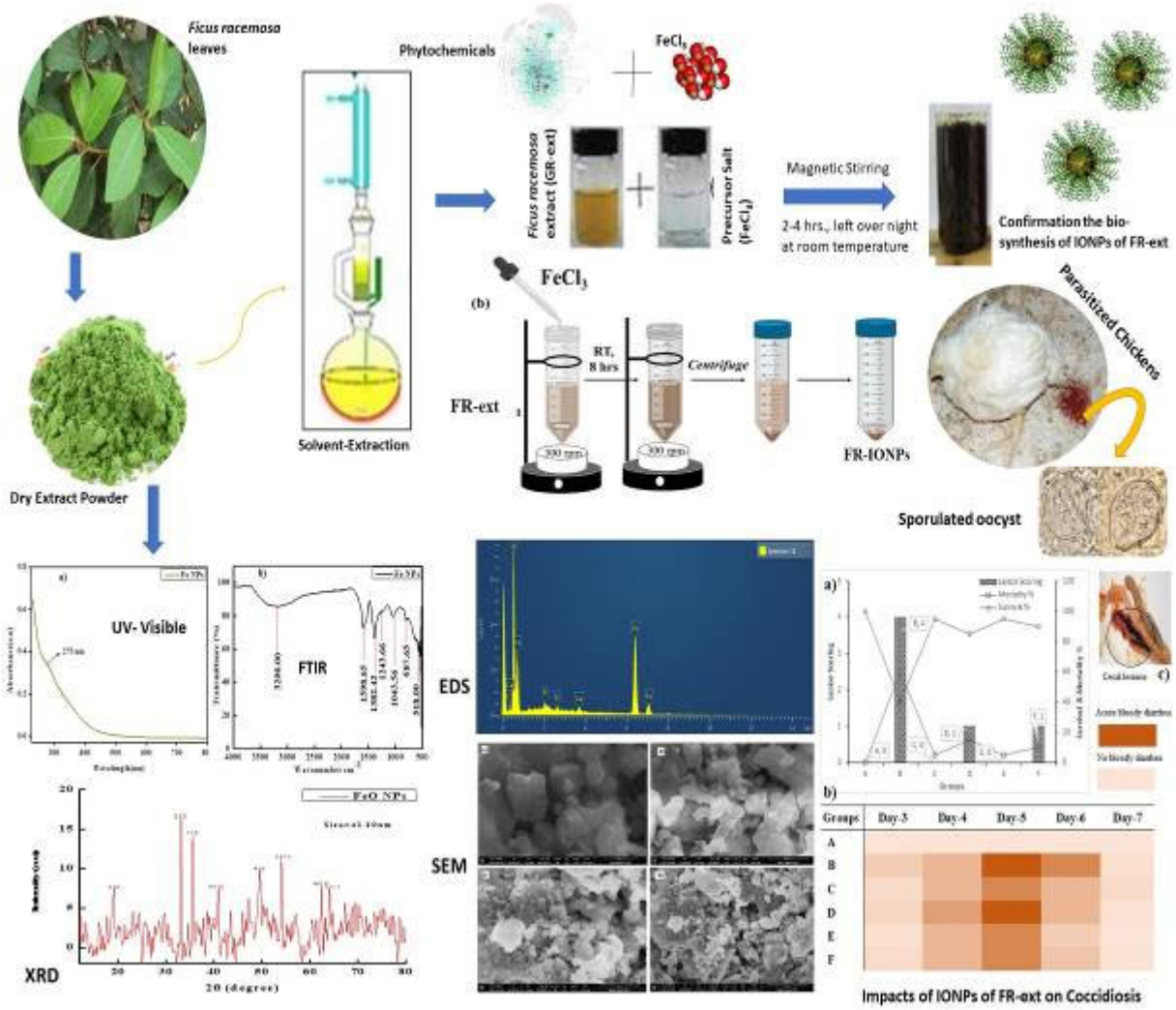
Results

The results indicated that IONPs at the moderate dose of 15 mg/kg significantly ($p < 0.05$) reduced the coccidial impacts by eliminating oocyst shedding per gram feces (up to 91%) and reducing clinical symptoms (lesions (LS = 0), bloody diarrhea (No), and mortality (0%)) in chicken at day 10 of treatment as compared to the negative control group-B (infected & non-treated). A dose-dependent and time-dependent trend were observed during treatments (10, 15, and 20 mg/kg) of 1-3 weeks using IONPs against the coccidial impacts on the growth parameters (body weight gain, mean feed consumption, feed conversion ratio) and biochemistry (plasma glucose, total protein, uric acid, ALT, AST, and ALP) in chickens. Additionally, *F. racemosa* IONPs at a dose of 15 and 20 mg/kg significantly recovered the parasitized and highly damaged hepatocytes, liver tissues, and ceca tissues after 1-3 weeks of treatment in broiler chickens. Our findings revealed that green synthesized IONPs at the concentration of 15 mg/kg play an important role in the recovery and growth enhancement in coccidiodized broiler chickens.

Conclusion

Therefore, the green synthesis of IONPs using leaf extract of *F. racemosa* could be a potential and safe anticoccidial agent with targeted implications in the poultry industry.

Fig. 1


Ficus racemosa leaves
Phytochemicals
FeCl3
Ficus racemosa extract (GR-ext)
Precursor Salt (FeCl3)
Magnetic Stirring
2-4 hrs., left over night at room temperature
Confirmation the bio-synthesis of IONPs of FR-ext
Solvent-Extraction
Dry Extract Powder
FeCl3
(b)
FR-ext
RT, 8 hrs
Centrifuge
FR-IONPs
Parasitized Chickens
Sporulated oocyst
UV- Visible
FTIR
EDS
SEM
XRD
FeO NPs
Impacts of IONPs of FR-ext on Coccidiosis

A14

Differential affinity chromatography coupled to mass spectrometry: a 3R-relevant tool to identify common binding proteins of a broad-range antimicrobial peptide and potentially other compounds active against *Toxoplasma gondii*

J. Müller[1], G. Boubaker[1], D. Imhof[1], K. Haenggeli[1], N. Haudenschild[1], L. M. Ortega-Mora[2], A. Hemphill[1]

[1]Institute of Parasitology, University of Bern, Vetsuisse Faculty, Bern, Germany
[2]University Complutense of Madrid, Saluvet, Madrid, Spain

Introduction: Leucinostatins and derivatives are antimicrobial peptides (AMPs) with broad-range of activities against cancer cells and infectious agents. One of these, ZHAWOC_6027 (AMP6027), was previously reported as a promising treatment option for African Sleeping Sickness (Brand et al. Angew Chem Int Ed Engl 2021, 60)

Objective: To investigate the efficacy of AMP6027 against *Toxoplasma gondii* in vitro and in vivo, and to identify potential drug targets.

Materials & methods: In vitro efficacy (EC50) and ultrastructural studies were done using *T. gondii*tachyzoites grown in human foreskin fibroblasts (HFF). For in vivo studies, AMP6027 (3 mg/kg/day for 5 days) was applied s. c. in mice experimentally infected with *T. gondii* oocysts, and cerebral parasite load was determined by real time PCR. The effects of peptide 6027 on B and T cells were investigated in murine splenocytes. To identify peptide 6027-binding proteins in *Toxoplasma* and splenocytes, differential affinity chromatography coupled to mass spectrometry and proteomics (DAC-MS-proteomics) was performed with cell-free extracts of *T. gondii* tachyzoites and mouse spleens using AMP6027 or an ineffective analogue.

Results: AMP6027 was highly efficacious in vitro (EC50 = 2 nM), and induced distinct mitochondrial changes as seen by TEM. HFF EC50 was 1000 x higher. In vivo, however, AMP6027 exacerbated the infection, caused mild clinical signs and elevated cerebral parasite load. AMP6027 also impaired the proliferation and viability of LPS-stimulated B cells *in vitro.* Following DAC, MS and proteomics of *T. gondii* extracts, 269 proteins were identified binding specifically to AMP6027, while in eluates from mouse spleen extracts 645 proteins specifically binding to this peptide were detected. Both datasets contained proteins involved mitochondrial energy metabolism and in protein processing and secretion, and in *T. gondii*, the cytochrome oxidase (TgApiCox25) complex was identified as the main molecular target among others These results suggest that AMP6027 interacts with common targets involved in essential pathways.

Conclusions: Since this methodology can be applied to various compounds as well as target cell lines or organs, DAC-MS-proteomics should be considered a smart and 3R-relevant way to (i) define putative drug targets in pathogens, and (ii) eliminate compounds that are prone to potential side effects before performing tedious and costly safety and efficacy assessments in animals or humans.

Fig. 1

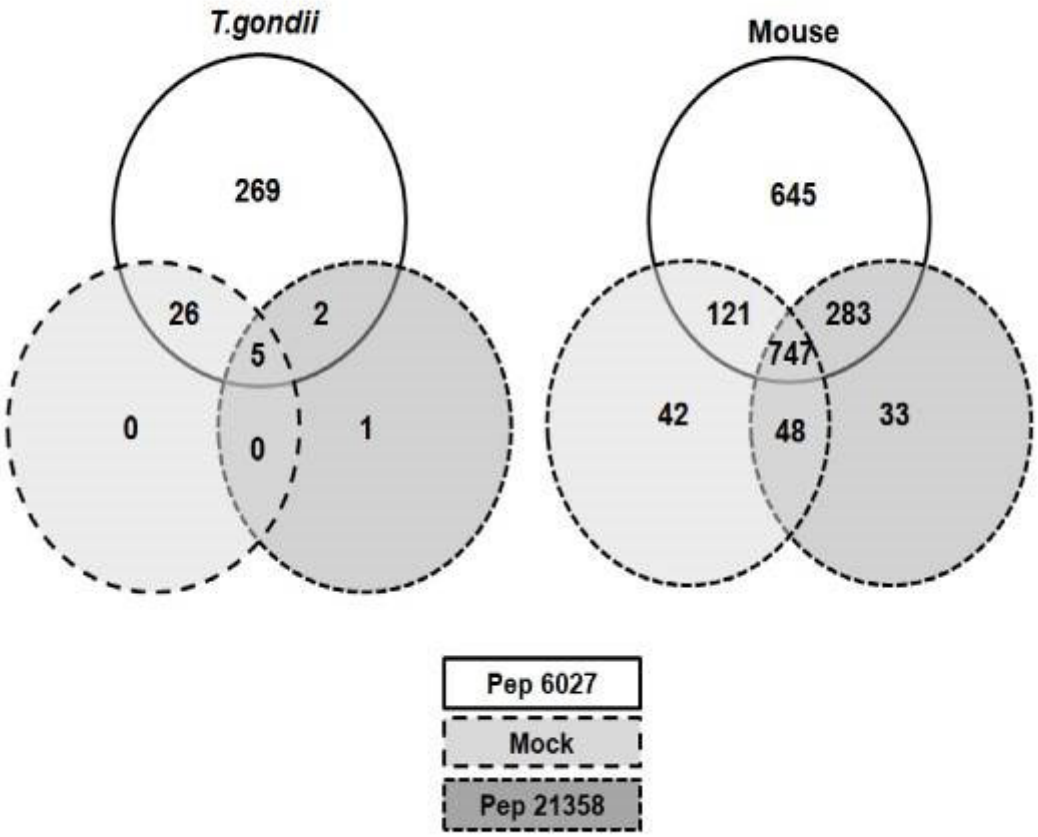
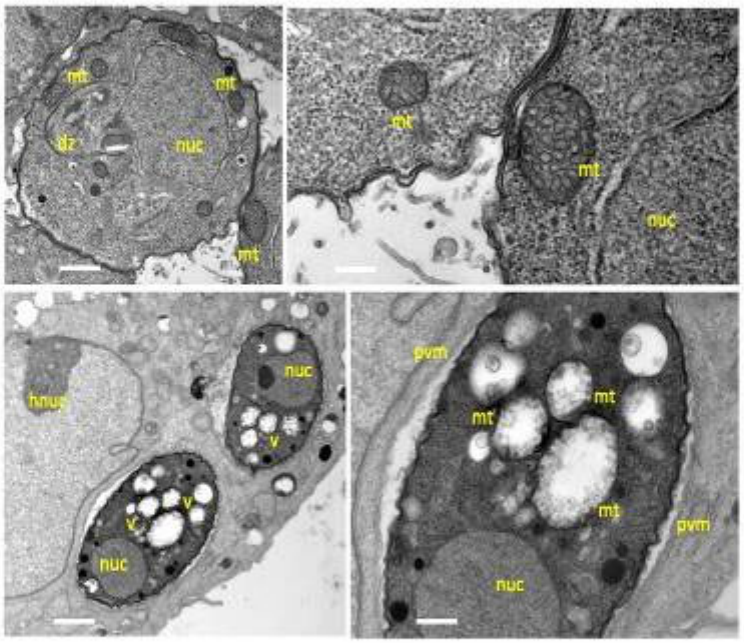

T.gondii
269
26
2
5
0
0
1
Mouse
645
121
283
747
42
48
33
Pep 6027
Mock
Pep 21358

Fig. 2



A15

Quantification of the metabolic activity of *Ascaris suum* L3 using resazurin reduction

A. Kundik[1], *S. Hartmann*[1], *F. Ebner*[1]

[1]Freie Universität Berlin, Department of Veterinary Medicine, Berlin, Germany

Helminth infections are a major health burden of humans and animals alike. Due to rising resistances against anthelmintics, there is an urgent need for new drugs. Current approaches for anthelmintic drug screening are considered subjective, laborious, and low in throughput since their endpoint readouts have to be evaluated microscopically. Here, we aimed to establish and optimize a fluorometric-based assay using resazurin and evaluated the assay by studying the metabolic activity of *Ascaris suum* (*A. suum*) larvae (L3), a highly prevalent parasite in swine.

A. suum L3 were assessed for their potential to reduce the non-fluorescent resazurin to the highly fluorescent resorufin using up to 1000 L3 and resazurin concentrations up to 15µg/ml. The assay was carried out in 96-microwell plates and fluorescence intensities were measured 24h post-incubation with the dye. Additionally, fluorescence microscopy was used to assess the resazurin reduction site within larvae. Finally, the assay was evaluated with larvae that have been exposed to different metabolic stressors or anthelmintics.

Our data show that resazurin is reduced in vital *A. suum* L3 to resorufin and released into the media. An intact L2 sheath around the L3 of *A. suum* completely prevented the uptake of resazurin while in unsheathed L3 a fluorescence signal was detected along the intestine. L3 which were metabolically affected by methanol or heat exposure showed a gradually decreased resazurin reduction activity. Exposure to ivermectin at 0.625µM, mebendazole at 5µM and thiabendazole from 10µM to 100µM for 24h significantly decreased larval resazurin reduction activity by 55%, 73% and 70% to 89%, respectively.

Together, our data show that metabolic stressors or anthelmintic drugs significantly reduce the resazurin reduction activity of *A. suum* L3, making the proposed assay a sensitive and easy to use high-throughput method to measure larval metabolic activity *in vitro*.

A16

Role of intrinsic factors in a long-term evolutionary ivermectin resistance study in the parasite model *Caenorhabditis elegans*

J. Hellinga[1], B. Trubenova[2], J. Wagner[1], A. Piecyk[3], R. Regoes[2], H. Schulenburg[3], J. Krücken[1], G. von Samson-Himmelstjerna[1]

[1]Freie Universität Berlin, Berlin, Germany
[2]Eidgenössische Technische Hochschule Zürich, Zürich, Switzerland
[3]Christian-Albrechts-Universität zu Kiel, Kiel, Germany

Introduction:

Free-living nematodes such as *Caenorhabditis elegans* (*C. elegans*) are an appealing tool for experimental evolution, given the easiness of working with them in cultured laboratory conditions. *C. elegans* is also a critical model for parasitic nematodes working with antiparasitic drugs, from understanding current and new drugs' molecular mechanisms to uncovering more drug targets. This is critical to counter the increase in antiparasitic drug resistance. However, the research of antiparasitic drugs with *C. elegans* has not been investigated from an evolutionary aspect.

Objective:

We present an evolution experiment detailing the impact of intrinsic factors such as population size and genetic diversity on the rate of ivermectin resistance evolution.

Methods:

We used a step-wise ivermectin introduction experiment to evolve resistant strains, which a mathematical model accompanied. The starting ancestor and final ivermectin-resistant populations were subjected to transcriptomic analysis by RNASeq and larval development assays with various anthelmintics.

Results:

We found that having a genetically diverse population shortened the time it took for the worms to evolve resistance to ivermectin. Different population sizes directly correlated to the rate of ivermectin resistance during the evolutionary experiment. This correlation was also predicted by mathematical modeling. Our RNASeq analysis is ongoing, yet we have many differences in gene expression between the ancestor and resistant worm populations. Lastly, our ivermectin-resistant populations were still sensitive to monepantel and albendazole but resistant to moxidectin and unexpectedly also to emodepside.

Conclusion:

The work done here is the first of many examples using evolution experiment guidelines to find answers for understanding which factors could influence the occurrence of anthelmintic resistance. We found that intrinsic factors such as genetic diversity and population size play a definitive role in the rate of ivermectin resistance formation in our evolutionary experiment. Our post-evolutionary experiments found that our ivermectin-resistant populations are also resistant to other macrocyclic lactones yet sensitive to other classes of anthelmintic drugs.

A17

Faecal egg count reduction tests and nemabiome deep sequencing data reveal widespread resistance against benzimidazoles and macrocyclic lactones in sheep but not cattle parasitic nematodes in north-east Germany

P. Ehnert[1], J. Krücken[1], S. Fiedler[2], F. Horn[2], C. Helm[1], S. Ramünke[1], T. Bartmann[1], A. Kahl[1], A. Neubert[2], W. Weiher[2], R. Daher[2], W. Terhalle[2], A. Klabunde-Negatsch[2], S. Steuber[2], G. von Samson-Himmelstjerna[1]

[1]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany
[2]Federal Office of Consumer Protection and Food Safety, Berlin, Germany

Introduction

Anthelmintic resistance (AR) of strongyle nematodes against all available drug classes is a worldwide problem in small ruminants and horses and is rapidly spreading in cattle. Particularly, multi-drug resistant parasites pose a high risk for animal health and production. However, since most strongyle eggs are not distinguishable, it is difficult to identify the particular species that has evolved drug resistance.

Objectives

Since no recent data on the occurrence of AR in German ruminants are available, the faecal egg count reduction test (FECRT) and deep amplicon sequencing (nemabiome) were used to identify resistant strongyle populations and species.

Materials and methods

The FECRT was conducted using Mini-FLOTAC with fenbendazole (FEB), ivermectin (IVM) and moxidectin (MOX) on eight, eight and twelve sheep farms, respectively. Susceptibility to FEB and eprinomectin (EPR) was investigated with FLOTAC on eight and seven cattle farms, respectively. The FECR with 95% confidence intervals was calculated with the R package eggCounts. Internal transcribed spacer 2 PCR products with 5'-Illumina *adapters* were barcoded and sequenced on a MiSeq (V3 2x300). Reads were assigned to strongyle species using the nemabiome database and species composition was calculated.

Results

In sheep, resistance was found on 6/8 farms for FEB, 6/8 farms for IVM, 3/12 farms for MOX and 3/8 farms for all three drugs. Nemabiome data were obtained for eleven farms before treatment and *Teladorsagia circumcincta* (5.8-91.1% of the larvae) was found on all of them. *Trichostrongylus colubriformis* (≤41.6%) and *Haemonchus contortus* (≤42.1%) occurred on 10/11 farms. *Chabertia ovina* (51.5%) and *Oesophagostomum venulosum* (27.3%) represented the most abundant species on one farm each. On three farms, triple resistant *H. contortus* and *T. circumcincta* were detected. Resistance to FEB and IVM was detected in *T. colubriformis*, *Trichostrongylus vitrinus*, *Trichostrongylus axei*, *Cooperia curticei* and *Cooperia fuelleborni*. In cattle, FEB was fully active on all eleven farms while EPR resistance was detected on 1/7 farms. *Ostertagia ostertagi* and *Cooperia oncophora* were the most abundant species but *Bunostomum phlebotomum* and *T. axei* were the most abundant species on one farm each. No post treatment nemabiome data were obtained.

Conclusions

In sheep, highly pathogenic parasite species with triple resistance occurred on multiple farms while anthelmintics in cattle were still fully active on most farms.

A18

Spatial Transcriptomics of parasites – 2D transcriptome analyses of tissues in the liver fluke *Fasciola hepatica*

S. Gramberg[1], *O. Puckelwaldt*[1], *T. Schmitt*[1], *S. Häberlein*[1]

[1]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany

Introduction

Fasciolosis is a food-borne trematode infection, caused by the liver fluke *Fasciola hepatica* and related species. The disease has considerable impact on human and animal health worldwide and costs the global livestock industry several billions of dollars per year. Limited therapeutic options and increasing anthelminthic resistance complicate sustainable control and highlight the need for novel anthelmintics.

Objectives

In order to identify new drug targets, it is first necessary to better understand the fluke"s biology, including its organ function and organ-specific gene expression. The cutting-edge technology *Spatial Transcriptomics* (ST) allows studying gene expression in a new dimension.

Material & Methods

Here, we applied Visium Spatial Gene Expression Solution (10x Genomics) on adult *F. hepatica*.

Results

Our ST data enables us to visualize gene expression in 2D, according to the original morphological context. This provides insights into tissue-specific gene expression throughout the fluke"s body. Gene expression profiles for eight different tissues, such as intestine, tegument and reproductive organs were identified and selected marker genes were validated by *in situ* hybridization. Gene ontology (GO) enrichment analysis revealed characteristic biological processes and molecular functions associated with each cluster. Finally, some genes with interesting spatial expression patterns were selected for functional characterization using RNA interference.

Conclusion

Taken together, this work provides the first spatial transcriptome of a parasitic flatworm. The dataset facilitates the design of further experiments that aim at characterizing tissue-specific gene function. Some of these genes may serve as new potential drug targets to better treat fasciolosis in the future.

A19

Three species of foodborne trematodes: a comparative RNAseq analysis of the hamster liver

E. Lishai[1,2], M. Pakharukova[1,2], O. Zaparina[2], S. J. Hong[3], B. Sripa[4], V. Mordvinov[2]

[1]Novosibirsk State University, Novosibirsk, Russian Federation
[2]Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russian Federation
[3]Convergence Research Center for Insect Vectors, Incheon National University, Incheon, South Korea
[4]WHO Collaborating Centre for Research and Control of Opisthorchiasis (Southeast Asian Liver Fluke Disease), Tropical Disease Research Center, Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

The foodborne trematodes *Opisthorchis felineus, O. viverrini* and *Clonorchis sinensis* induce liver fluke infections in mammals, including humans. The species have different carcinogenic potential: *O. viverrini* and *C. sinensis* are assigned to Group 1A of biological carcinogens, while *O. felineus* belongs to Group 3A.

To provide in-depth research and to gain insights into the species-specific mechanism by which liver fluke infections cause liver lesions we investigated differential gene expression of liver transcriptomes (Illumina HiSeq2500) of golden hamsters infected with *O. felineus, O. viverrini* and *C. sinensis* at 1 and 3 months postinfection.

The libraries contained 905,079,329 sequences (average 37,711,638.7 reads per sample). The STAR aligner, DESeq2, clusterProfiler and BisqueRNA R-packages were applied.

Principal components analysis revealed high clustering of samples by species and by the time in infection. In *C. sinensis* infection, the highest number (2886) differentially expressed genes (DEGs) were observed. Whereas *O. felineus* and *O. viverrini* infections change the expression of 1790 and 1501 DEGs, respectively.

Cluster analysis revealed that activated cellular pathways were different between acute and chronic infection. Transcriptomic data were supported by the results of western blotting, revealing the presence of fibrogenesis-related proteins. DEGs were enriched by common MSigDB signatures such as myogenesis, inflammatory response, late estrogen response, IL2 STAT5 signaling, TNFA signaling via NFKB for all three infections. Epithelial-to-mesenchymal transition, IL6 JAK STAT3 signaling, and TGFB signaling were represented in *C. sinensis* and *O. felineus*- infected hamster, but not after *O. viverrini* infection.

Common enriched KEGG pathways were Hippo, PI3K-Akt and calcium signaling pathways, while PPAR signaling pathway was present only in *C. sinensis* and *O. viverrini* infections. Significant interspecies differences in the response of cholangiocytes, stellate cells and M1 macrophages were found.

Our data provide knowledge about species-specific changes in gene expression in the liver fluke–infected host liver and contribute to understanding the biliary fibrosis and neoplasia associated with liver fluke infections. This work was supported by the Russian Science Foundation [grant number 22-24-20010].

A20

Mass spectrometry-based analysis of proteome and lipidome reveals liver metabolic reprogramming induced by *Schistosoma mansoni* infection

P. Ghezellou[1], S. Gerbig[1], K. R. Wiedemann[1], D. Luh[1], M. Roderfeld[2], E. Roeb[2], C. G. Grevelding[3], B. Spengler[1]

[1]Justus-Liebig-University Gießen, Institute of Inorganic and Analytical Chemistry, Gießen, Germany
[2]Justus-Liebig-University Gießen, Department of Gastroenterology, Gießen, Germany
[3]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany

Introduction

Schistosomiasis is a neglected tropical disease estimated to affect at least 236 million people from 78 countries. However, the underlying mechanisms of intestinal and hepatic schistosomiasis caused by the species *Schistosoma mansoni* are complex and still not fully elucidated. Mass spectrometry (MS) as an analytical technique is becoming increasingly important in bioscience, quickly analyzing the molecular composition of a sample, helping to determine the chemical composition and addressing complex biological problems.

Objectives

We investigated the hepatic proteome and lipidome composition of *S. mansoni* infected hamsters. We aim to uncover differentially regulated hepatic constituents in the context of *S. mansoni* infection to identify characteristically altered key molecules involved in hepatic metabolism modulation.

Materials & Methods

We applied MS-based accurate quantitative proteomic and lipidomic analysis to define molecular pathways involved in hepatic schistosomal infection. We also subjected liver tissue of infected hamsters to high-resolution AP-SMALDI mass spectrometry imaging (MSI) to visualize the lipid distribution in tissue sections with high lateral resolution.

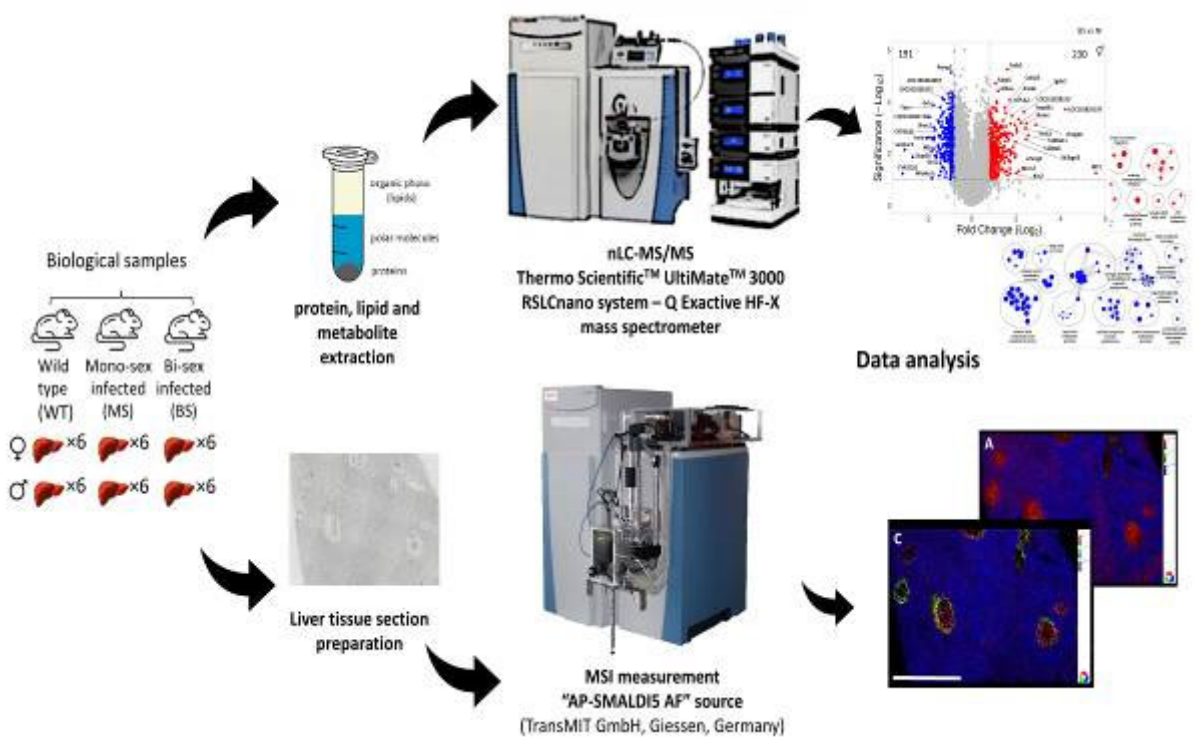
Results

The disease provoked enormous alterations in the expression of liver proteomes and lipidomes. In the infected livers, the regulated molecules are involved in various biological functions such as immune response, cytoskeleton reorganization, apoptotic signaling, energy-generating, and a broad range of biosynthetic/metabolic processes. We also characterized several markers specific to the infection by AP-SMALDI MSI and exhibited their distribution in the tissue sections.

Conclusion

The mass spectrometric analysis of the liver samples indicates a characteristic regulation of the hepatic metabolism during schistosomiasis. Our findings expand the knowledge about biological pathways that are regulated in the liver upon *S. mansoni* infection and furthermore provide information that may also serve for diagnostic purposes. In summary, the combination of MS techniques used in our study demonstrates the potential of this combined approach to enlarge our understanding of the complex pathologies caused by host-parasite interactions.

Fig. 1



A21

Liver-dependent protective immune responses against *Ascaris* infections

L. E. Elizalde Velázquez[1], *J. Schlosser-Brandenburg*[1], *S. Hartmann*[1]

[1]Freie Universität Berlin, FB Veterinärmedizin, Institut für Immunologie, Berlin, Germany

Introduction: The liver is an effected organ of different parasites whether it is protozoan or helminth infections. Even so for both human and porcine *Ascaris* infections, which affect > 0.7 billion humans in the global south and lead to huge economic losses in the pig farming, respectively. In fact, larval stages of *Ascaris* when migrating through the liver are taking advantage of the tolerogenic environment hepatic tissue provides. However, concomitantly, the formation of pathological lesions in the liver, known as white spots, also suggests a strong liver immune response and inflammation lead to elimination of the parasites during the initial phase of larval migration.**Objectives:** In this research project, we aim to determine whether resistance to *Ascaris* larval migration occurs in the liver and whether liver innate immune cells are involved in the elimination of *Ascaris* larvae or are targets of immunoregulation by the parasite.**Materials & methods:** To address this, we infected two different mouse strains with *Ascaris* that mimic hepato-tracheal migration of natural hosts but show contrasting susceptibilities to *Ascaris* infection. We immunologically analyzed liver innate immune cells such as innate lymphocytes, eosinophils, neutrophils and macrophages (MO) to determine their composition and phenotype during liver and lung stage of an *Ascaris* infection.**Results:** Our results show, resistant CBA mice harbor significantly less *Ascaris suum* larvae in the liver during liver stage of infection compared to susceptible C57BL/6 mice. Interestingly, hepatic resistance against *Ascaris* larval migration was associated with differences in polarization of liver MO. Already intrinsically, the frequencies of alternatively activated MO were elevated in the liver of the resistant strain in comparison to susceptible strain. Interestingly, *Ascaris* infection led to a hepatic downregulation of this host protective M2 macrophage response. Simultaneously, both mouse strains showed increased iNOS expression in liver MO which suggest larval clearance might be partly associated with a type 1 response but not being an effective host protective response as in the susceptible strain significant amounts of larvae were detectable despite iNOS production.**Conclusion:** Alternative activation of MO in liver seems to be associated with resistance and being actively counterregulated by infection. In contrast, iNOS production of liver macrophages is induced but ineffective in *Ascaris* larval killing.

A22

Highly functional Th2 effector cells coexist with IFN-g competent Th2/1 hybrid cells in the liver of nematode infected mice

J. Adjah[1], B. Kaspe[1], S. Hartmann[1], S. Rausch[1]

[1]Freie Universität Berlin, Veterinary Medicine, Berlin, Germany

Introduction

The small intestinal nematode *Heligmosoides polygyrus* establishes chronic infections in most mouse lines despite the induction of strong Th2 responses. We previously identified IFN-g competent Th2/1 hybrid cells as confounders of effective type 2 immunity in *H. polygyrus* infected mice and showed recently that age- and genotype-dependent differences in resistance to the infection correlate with the proportion of Th2/1 hybrid cells in nematode-induced CD4+GATA-3+ T cells.

Objectives

Th2/1 hybrid cells are relatively rare in the gut draining lymph nodes but may account for about half of the GATA-3+ effector cells in spleen and blood. We therefore investigated which other sites might serve as sources for nematode induced Th2/1 cells.

Materials and methods

We used the *Heligmosoides polygyrus* model for studying immune responses to helminth in C57BL/6 mice. A multi-color flowcytometric technique, as well as other enzyme-linked assay was also used to investigate the expression of type 1 and 2 immune markers on T cells after *ex vivo* cultures.

Results

We observed large numbers of GATA-3+ cells accumulating in the liver early during infection. These were enriched in Th2/1 cells, maintained during memory formation, and rapidly reactivated upon challenge infection in drug cured mice. Fitting the strong hepatic accumulation of CXCR3+ Th2/1 cells, myeloid cells isolated from the liver of infected mice stood out in the spontaneous production of the CXCR3-ligand CXCL9.

Conclusion

Together, these data suggest that IFN-g released early during worm infection results in the induction and systemic accumulation of IFN-g-competent type 2 effector cells. Whether spleen and liver limit excessive hybrid responses via apoptosis or benefit from Th2/1 accumulation in the surveillance of microbial translocation in enteric nematode infection is under current investigation.

A23

The Achilles' heel of the fox tapeworm? – Investigation of the threonine metabolism of *Echinococcus multilocularis*

M. Kaethner[1,2], P. Zumstein[1], M. Preza[1], P. Grossenbacher[2], M. Lochner[2], S. Schürch[3], C. Regnault[4], D. Villalobos Ramírez[5], B. Lundström-Stadelmann[1,6]

[1]University of Bern, Institute of Parasitology, Vetsuisse Faculty, Bern, Switzerland
[2]University of Bern, Graduate School for Cellular and Biomedical Sciences, Bern, Switzerland
[3]University of Bern, Department of Chemistry, Biochemistry and Biochemistry, Bern, Switzerland
[4]University of Glasgow, Glasgow Polyomics, College of Medical, Veterinary and Life Sciences, Glasgow, United Kingdom
[5]University of Würzburg, Department of Bioinformatics, Würzburg, Germany
[6]University of Bern, Multidisciplinary Center for Infectious Diseases, Bern, Switzerland

Introduction
Alveolar echinococcosis (AE) is a severe zoonotic disease caused by the metacestode of the fox tapeworm *Echinococcus multilocularis*. Novel treatment options are urgently needed. *In vitro* experiments showed strong scavenging of threonine by *E. multilocularis* metacestodes. Metacestodes express a threonine dehydrogenase (EmTDH) that is potentially a future drug target against AE, as human TDH is non-functional.

Objectives
We aimed to investigate the importance of threonine uptake and metabolism for *E. multilocularis* and to study whether threonine-degrading pathways can be targeted for the development of new drugs against the parasite. Quinazoline carboxamids (QCs) are potent inhibitors of mouse TDH and we aimed to test them against *E. multilocularis.*

Material and methods
$^{13}C_4$ L-threonine and metabolites were traced in *in vitro* cultured metacestodes to give insights into the metabolization of threonine in *E. multilocularis*. In addition, we studied the effect of L-threonine on the growth of *in vitro* cultured metacestodes and on the vesicle formation of *E. multilocularis* primary cells. QCs were tested against *E. multilocularis* metacestodes via damage marker release assay and in enzymatic assays against EmTDH activity in crude extracts of metacestodes as well as recombinantly expressed EmTDH.

Results
Flux analysis with $^{13}C_4$ L-threonine showed that *E. multilocularis* metacestodes take up threonine and metabolize it to glycine, which is indicative for a functional TDH pathway. Significantly increased growth was shown for threonine-treated metacestodes. Increased numbers of vesicles were formed upon treatment of primary cell cultures with threonine, whereas the threonine analogue 3-hydroxy norvaline reduced the number of vesicles. One QC inhibited EmTDH in an enzymatic assay using crude extract of metacestodes and it was toxic against *E. multilocularis* metacestodes. However, recEmTDH was not inhibited by any of the here tested QCs.

Conclusion
Flux analysis, growth assays and vesicle formation assays provided evidence that threonine metabolism is important for *E. multilocularis* and inhibiting this metabolism could provide new treatment options. Further investigation of the *in vivo* situation are needed. Novel QC derivatives will be designed for successful inhibition of EmTDH to be tested in the here established enzymatic assays for medium throughput screening of potential other inhibitors targeting the threonine metabolism of *E. multilocularis*.

A24

Chromosomal integration of a reporter gene by RNA-guided Cas-enzymes into a predicted genomic safe-harbor site of *Schistosoma mansoni*

M. F. Möscheid[1], P. Wistiphongpun[2], W. Ittiprasert[2], T. Quack[1], V. H. Mann[2], C. Grunau[3], P. J. Brindley[2], C. G. Grevelding[1]

[1]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany
[2]George Washington University, Department of Microbiology, Immunology & Tropical Medicine, & Research Center for Neglected Diseases of Poverty, School of Medicine & Health Sciences, Washington, DC, United States
[3]University of Perpignan Via Domitia, IHPE Laboratory for Host-Pathogen-Environment Interactions, Perpignan, France

"Introduction"

Schistosoma mansoni, a zoonotic parasite, is characterized by a complex lifecycle that includes an invertebrate snail host and a vertebrate final host. Until now, no established protocol exists for the stable transformation of schistosomes.

For investigating the function of a gene of interest (GOI), knock-out models are common for various organisms, but not yet available for trematodes. Until now, RNA interference (RNAi) has been proven as the most suitable method for functional gene characterization. However, RNAi efficiency varies, and it can lead to ectopic effects. CRISPR/Cas-based editing is a powerful tool for gene characterization. To make this technique accessible for trematode research, we tried to establish a protocol for editing a bioinformatically predicted genomic safe harbor site (GSH) of *S. mansoni*. GSHs are sites in the genome that allow the integration of new genetic material without negatively effecting genome integrity or gene expression.

"Objectives"

The aim of this project was to establish a CRISPR/Cas-based approach to generate transgenic *S. mansoni*.

"Material and methods"

For editing the identified GSH, a 5'C6-PEG10-modified construct encoding an eGFP reporter-gene driven by a strong native promoter was used as donor DNA. Cas-mediated integration of the transgene was performed by electroporation of eggs. To this end, ribonucleoprotein complexes (RNPs) were used consisting of the enzymes Cas9 or Cas12a and respective guide RNAs targeting the GSH. Integration of the transgene was examined by PCR. Reporter-gene expression was analysed by RT-PCR at the transcript level, and by confocal laser scanning microscopy to reveal eGFP expression.

"Results"

Using both Cas9 and Cas12a, we demonstrated the accessibility of the predicted GSH. Comparison of both Cas-enzymes revealed a significantly higher editing efficiency for Cas12a. Furthermore, we confirmed reporter-gene integration into the selected GSH using both RNPs formed by Cas9 or Cas12a. Finally, eGFP signals were detected demonstrating reporter gene expression in electroporated eggs and miracidia.

"Conclusion"

Successful editing of a predicted schistosomal GSH was shown as well as the expression of a genome-integrated eGFP reporter-gene. These results are proof of concept for a new genome-editing approach in *S. mansoni*. Our results suggest that both enzymes, Cas9 and Cas12a, are useful for editing the *S. mansoni*, with a higher efficiency for Cas12a.

A25

Application of an RNA-seq-based method for the global analysis of self-cleaving ribozyme activity in *Schistosoma mansoni*

V. J. Olzog[1], J. L. Fournes[1], M. E. Überall[2], C. G. Grevelding[2], J. Fallmann[3], C. E. Weinberg[1]

[1]Leipzig University, Institute of Biochemistry, Leipzig, Germany
[2]Justus-Liebig-University Gießen, Institute of Parasitology, BFS, Gießen, Germany
[3]Leipzig University, Department of Computer Science and Interdisciplinary Center for Bioinformatics, Leipzig, Germany

Ribozymes are RNAs that catalyze chemical reactions. Some ribozymes, so-called self-cleaving ribozymes, cut their own sugar phosphate backbone at a specific position to enable their biological functions. In the bloodfluke *Schistosoma mansoni* thousands of self-cleaving ribozymes are predicted. As their analysis by classic biochemical approaches is tedious, we know little about their activity *in vivo*.

We developed and validated a transcriptome-wide method, called cyPhyRNA-seq, to screen for active self-cleaving ribozymes in total RNA extracts. With this strategy, we successfully analyzed self-cleavage activity of more than 100 self-cleaving ribozymes from paired and unpaired schistosomes in a proof-of-principle study. We uncovered several intriguing ribozyme representatives: We found active ribozyme examples as part of mobile genetic elements such as Perere-3, W-elements and SINE retrotransposons. Furthermore, we found ribozyme sequences with mutations expected to inactivate them but that nonetheless function *in vivo*. For a representative of such ribozymes, we show *in vitro* evidence for a possible *trans*-cleavage mechanism, whereas all known natural self-cleaving ribozymes cleave in *cis*. Additionally, we identified differences in ribozyme activity between paired and unpaired schistosomes, suggesting a pairing-dependent expression and cleavage for some of the self-cleaving RNAs in *Schistosoma*. Lastly, we investigated a subset of the representatives *in vitro* using co-transcriptional cleavage assays and reverse transcription PCR to validate their cyPhyRNA-seq activity profile.

Thus, cyPhyRNA-seq is crucial for the monitoring of ribozyme activity in organisms that harbor self-cleaving RNAs and it facilitates the investigation of self-cleavage activity under different conditions, such as stress or developmental stage. Differences in ribozyme activity between investigated conditions could point to novel biological roles of these RNAs and might even suggest that self-cleaving ribozyme activity is regulated. Lastly, the application of cyPhyRNA-seq to a ribozyme-rich organism such as *Schistosoma* may enable the discovery of novel self-cleaving ribozymes. These research endeavors contribute to our current understanding of the versatility of RNA and its biological significance and enable a global, functional screening approach.

A26

Helminthic glutamate dehydrogenase-dependent PGE2 production in monocyte and microglia potentiates Treg development with distinct transcriptional and epigenetic profiles

F. U. Prodjinotho[1,2], V. Gres[3], F. Henkel[4], M. Lacorcia[1], R. Dandl[5], M. Haslbeck[5], V. Schmidt[6,2], A. S. Winkler[6,2], C. Sikasunge[7], P. J. Jakobsson[8], P. Henneke[3,9], J. Esser-von Bieren[4], C. Prazeres da costa[1,2,10]

[1]Institute for Medical Microbiology, Immunology and Hygiene, TUM, Munich, Germany
[2]Center for Global Health, TUM School of Medicine, Technical University of Munich (TUM), Munich, Germany
[3]Institute for Immunodeficiency, Center for Chronic Immunodeficiency, Medical Center and Faculty of Medicine, University of Freiburg, Freiburg i. Br., Germany
[4]Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Munich, Germany
[5]Technical University Munich (TUM), Department of Chemistry, Garching, Germany
[6]Department of Neurology, University Hospital, Klinikum rechts der Isar, Technical University Munich (TUM), Munich, Germany
[7]School of Veterinary Medicine, Department of Paraclinicals, University of Zambia, Lusaka, Zambia
[8]Rheumatology Unit, Department of Medicine, Solna, Karolinska University Hospital, Stockholm, Sweden
[9]Center for Pediatrics and Adolescent Medicine, Medical Center, University of Freiburg, Freiburg i. Br., Germany
[10]German Center for Infection Research (DZIF), Munich, Germany

Introduction: Immunoregulation of inflammatory, infection-triggered processes in the brain constitutes a central mechanism to control devastating disease manifestations such as epilepsy. In neurocysticercosis (NCC), an inflammatory and clinically pleomorphic disease of the human brain, and most common cause of epilepsy in endemic regions, the disease severity strongly depends on the viability of the larval cyst of the pork tapeworm *T. solium*. Whereas dead cysts are often associated with epileptic manifestations, viable cysts in the brain mostly remain clinically silent by yet unknown mechanisms, potentially involving regulatory T cells (Tregs) in controlling inflammation.

Objective: In this work, we aim to uncover the underlying mechanisms for this dichotomy, especially the nature of cyst products and mechanisms controlling the development of Tregs during asymptomatic NCC and inflammation during symptomatic NCC.

Materials and Methods: Peripheral and brain immune cells from mice and healthy volunteers were pulsed with parasite viable, decaying cyst materials and the recombinant expressed cyst enzyme glutamate dehydrogenase GDH. Immune modulation and underlying mechanistic aspects were identified via adoptive transfer of cyst-treated DCs, and qPCR/FACS surrogate markers expression associated with LC/MS/MS profiling of eicosanoids and precursors and PGE2/IL-10 receptors antagonists. The mechanisms underlying Treg development and epigenetic landscape and transcriptional signatures associated with GDH-PGE2/IL-10-induced Tregs as well as FACS-sorted Tregs from NCC infected and healthy individuals were addressed via sequencing (ATACSeq, RNASeq).

Results: We demonstrated that the enzyme GDH from parasite viable cyst instructs tolerogenic $CD206^{+}$ monocytes and Iba-1^{lo} microglia to release IL-10 and the lipid mediator PGE2. These act in concert via their respective receptors, converting naive $CD4^{+}$ T cells into brain homing $CD25^{hi}FoxP3^{+}CTLA\text{-}4^{+}CCR6^{+}CCR7^{+}$ Tregs with distinct transcriptional signatures (e.g. JAK-STAT pathway) as identified in asymptomatic NCC patients. Moreover, while viable cyst strongly upregulated IL-10 and PGE2 transcription in microglia leading to Treg development, dead cyst material lacking GDH enzyme induced proinflammatory non-phagocytic microglia and TGF-β as potential drivers of epilepsy.

Conclusion: Harnessing the GDH-PGE2-IL-10 axis and targeting TGF-ß signaling may offer an important therapeutic strategy in inflammatory epilepsy and NCC.

A27

Towards a cell atlas for the liver fluke *Fasciola hepatica*

O. Puckelwaldt[1], J. Koepke[2], A. Hoek[3], C. Samakovlis[2], A. Goesmann[3], S. Häberlein[1]

[1]Justus-Liebig-University Gießen, Institut for Parasitology, Gießen, Germany
[2]UKGM Gießen, Cardio-Pulmonary Institute, Gießen, Germany
[3]Justus-Liebig-University Gießen, Bioinformatics and Systems Biology, Gießen, Germany

The worldwide prevalent liver fluke *Fasciola hepatica* causes fascioliasis, a neglected tropical disease and zoonosis. Comprehensive knowledge on the parasite"s cell types and cell-specific gene expression repertoire is missing to date. This kind of insight would take research on drug target genes, developmentally, and metabolically important genes to a next level. Recent technological advances, like the introduction of microfluidics in single-cell RNA sequencing (scRNA-seq) have greatly enhanced and may be transferred to multicellular parasites. Here, we provide the first whole-organism cell atlas of *F. hepatica* as a basis for expression analysis of genes on a single-cell level.

We established a digestion protocol to obtain high-quality single-cell preparations from adult *F. hepatica* worms. Cells were then enriched by flow cytometry and finally 19.000 cells processed by 10X Genomics scRNA-seq technology. Cell clusters and marker genes were identified by using the software package Seurat in R. *In-situ* hybridization (ISH) was used to confirm the sites of gene expression. Gene ontology (GO) enrichment analysis revealed specific biological processes, molecular functions, or cellular components associated with the identified cell clusters.

We successfully identified more than 17 cell clusters that represent distinct cell types, including gastrodermal cells expressing cathepsins, somatic stem cells (neoblasts) expressing nanos2, and neuronal cells. Marker genes for each cluster were identified and their expression confirmed by ISH. Some clusters were enriched for genes of important biological processes, like signal transduction in neuronal cells and proliferation in the neoblast cluster. The resolution of this dataset allowed uncovering potential lineages spanning from the central neoblast cluster into other clusters like the vitellarium, spermatogonia and the female germline. Additionally, we were also able to identify a tissue-specific expression of several protein kinase genes in the tegument as well as reproductive organs, a gene family that is well studied in other helminths with respect to druggable targets.

We present the first transcriptome for the liver fluke *F. hepatica* on a single-cell resolution. This dataset can serve as treasure trove for the discovery and study of tissue-type and cell-type specific genes in this parasite.

A28

The Hippo pathway regulates *Echinococcus* stem cell function

L. Schiegl[1], M. Bergmann[1], K. Brehm[1]

[1]Universität Würzburg, Institut für Hygiene und Mikrobiologie, Würzburg, Germany

Introduction: Alveolar echinococcosis (AE) is a lethal zoonosis prevalent in the Northern Hemisphere and is caused by the metacestode larva of the fox tapeworm *Echinococcus multilocularis*. Growth of the Echinococcus metacestode within host organs is stem cell driven and almost unrestricted, leading to massive tumorous lesions. Organ size in metazoans is typically regulated by the Hippo pathway, the contribution of which to *Echinococcus* development has so far not been addressed. Objectives: To characterize *Echinococcus*Hippo signaling components and their contribution to parasite development. Materials & methods: We used bioinformatics to identify *Echinococcus* Hippo pathway components and in vitro cultivation systems, combined with inhibitor assays were used to study their role in *Echinococcus* proliferation and growth. Results: We identified *Echinococcus* orthologs of the kinases Hippo and Warts, the regulatory proteins Salvador and Mats, the co-activator Yorkie and the transcription factor Scalloped which are all expressed by differentiated cells and by stem cells in *Echinococcus* larvae. By applying the known Hippo inhibitors TRULI and XMU-MP-1 to metacestode vesicles and protoscoleces, we observed significant effects on larval morphology indicating alterations in stem cell proliferation. Particularly, XMU-MP-1 induced "giant" stem cells in metacestode vesicles, indicating direct influences of Hippo signaling on parasite mitosis. Furthermore, we show that RNAi knockdown of *Echinoccocus* yorkie induces vesicle formation by stem cells. Conclusions: Our data indicate that a complete Hippo-signaling pathway is present in *E. multilocularis* and contributes to proliferation dynamics of germinative stem cells, which are the decisive cell type in parasite growth within the host. Our data are relevant for understanding Echinococcus stem cell based developmental processes and may be exploited for the development of novel anti-parasitics.

A29

Genetic structure and phylogeography of *Echinococcus granulosus* sensu stricto genotypes and potential role of buffalo-dog cycle in CE epidemiology in Pakistan

N. Mehmood[1,2], H. Muqaddas[3], M. I. Ullah[4]

[1]University of Sassari, Veterinary Medicine, Sassari, Italy
[2]University of Sargodha, Zoology, Sargodha, Pakistan
[3]The Women University Multan, Zoology, Multan, Pakistan
[4]University of Sargodha, Entomology, Sargodha, Pakistan

Introduction

Pakistan is a neglected endemic focus for *Echinococcus granulosus* sensu lato, a zoonotic parasite species complex with the ability to infect wide spectrum of hosts. Wide gaps exist in literature for etiological agents of cystic echinococcosis (CE) involved in epidemiological manifestation of this disease.

Objectives

The study explored main transmission routes of this disease through molecular characterization of hydatid cyst isolates.

Materials & methods

The hydatid cysts were collected from the seven major cities, encompassing four provinces, of Pakistan. Molecular characterization of the isolates obtained from sheep (n = 35), goats (n = 26), cattle (n = 30) and buffalo (n = 30) was based on partial mitochondrial *nad1* gene. Additionally, computation of different genetic diversity indices such as number of haplotypes (hn), nucleotide diversity (nd) and haplotype diversity (hd) was carried out. The four subpopulations of *E*. *granulosus* were also evaluated for neutrality indices and estimation of genetic differentiation and degree of gene flow by employing a pairwise fixation index (Fst).

Results

Two strains of *E*. *granulosus sensu stricto*, G1/G3, and their haplotypes (n = 9) were observed to be cycling in sympatry in the domestic ungulate populations. G3 genotype had higher prevalence (66.94%) in the hosts compared to G1 genotype (33.06%) which was not surprising, considering the large buffalo population in Pakistan. Elucidating local transmission patterns of *E*. *granulosus* sensu stricto, buffalo-dog cycle emerged as one of the dominant causes of G3 dispersal. Population diversity indices had overall moderate values of haplotype diversity (0.6285 ± 0.038) coupled with low nucleotide diversity (0.00271 ± 0.0002). Population demographics and genetic variability indices suggested expanding parasitic population in multiple host spectrum.

Conclusion

It was demonstrated that the *E. granulosus* s.s. population in Pakistan was highly differentiated from the other regions of the world. Furthermore, large population of buffaloes in this region and stronger affinity of G3 for this host reflect heterogeneity in host specificity and adaptability to local environmental conditions making buffalo-dog cycle as one of the major transmission routes in CE epidemic in Pakistan. Following other regional studies, where sheep-dog cycle is considered as the major source of disease, might not fit the epidemiological situation in South Asia.

Fig. 1

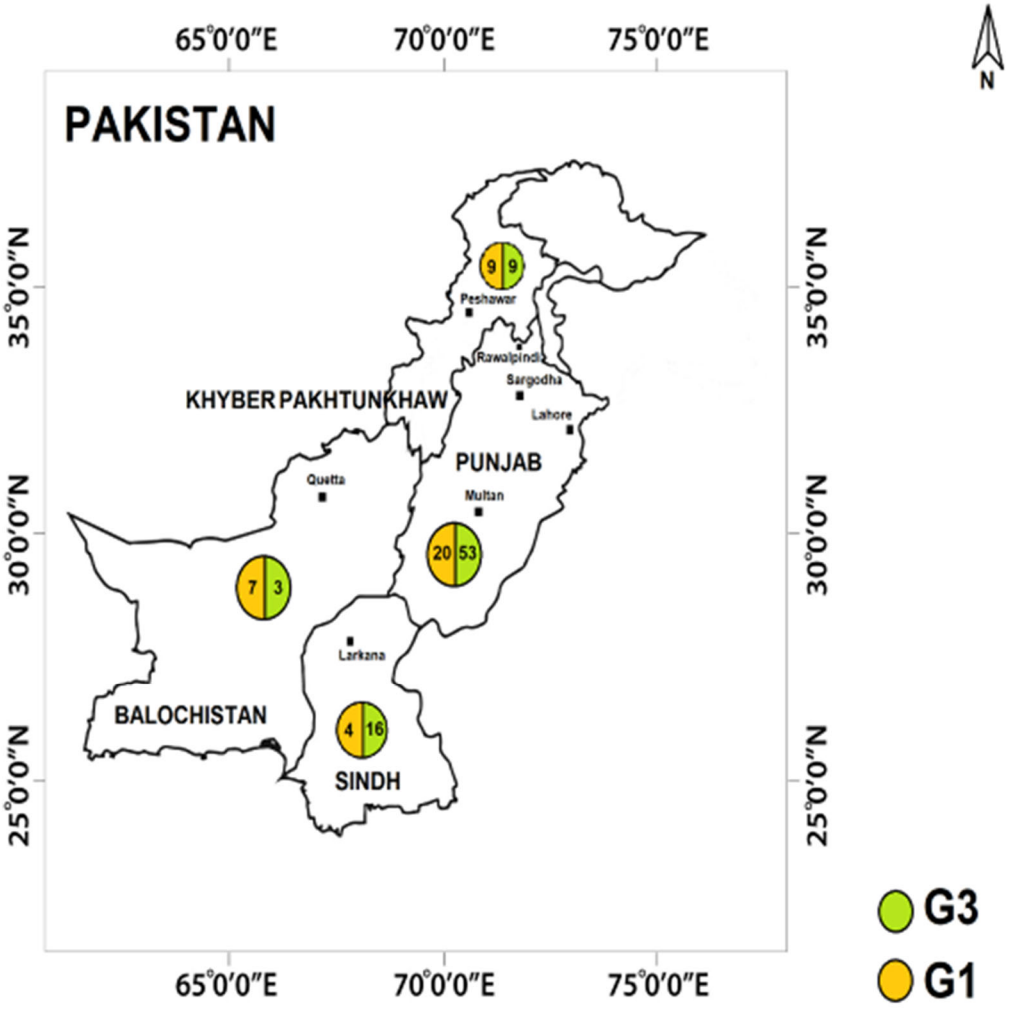
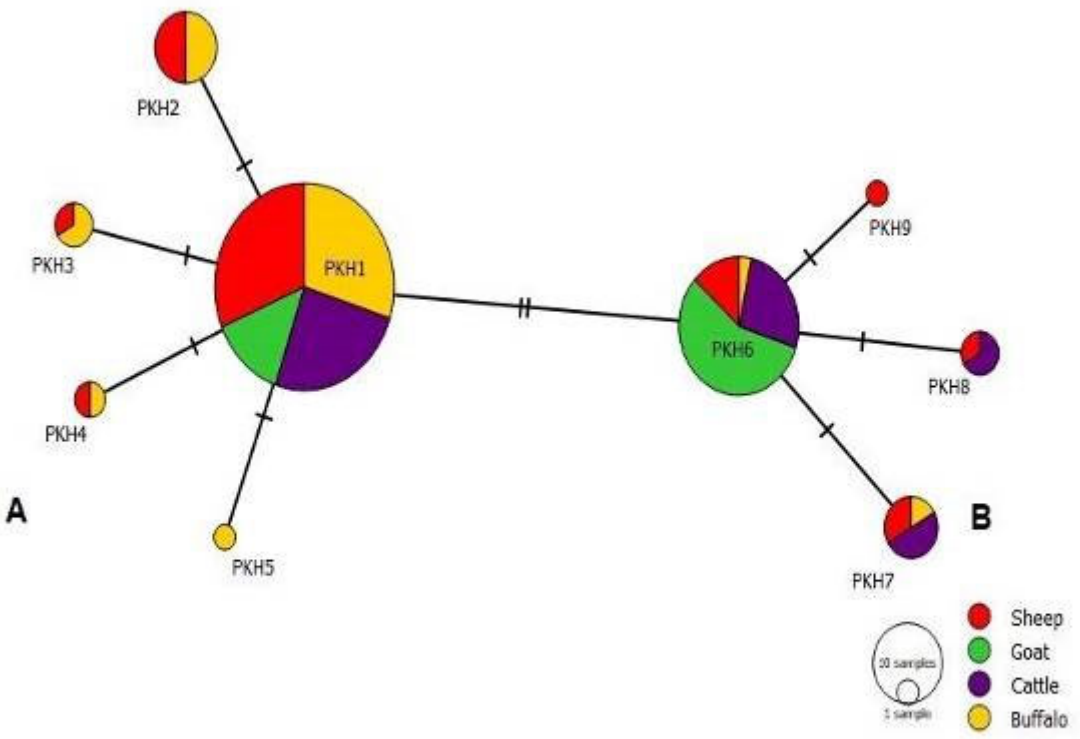

65°0′0″E
70°0′0″E
75°0′0″E
35°0′0″N
30°0′0″N
25°0′0″N
N
PAKISTAN
KHYBER PAKHTUNKHAW
PUNJAB
BALOCHISTAN
SINDH
Peshawar
Rawalpindi
Sargodha
Lahore
Multan
Quetta
Larkana
9
9
20
53
7
3
4
16
G3
G1

Fig. 2


PKH2
PKH3
PKH1
PKH4
PKH5
A
PKH9
PKH6
PKH8
PKH7
B
Sheep
Goat
Cattle
Buffalo

Session V – Veterinary Parasitology & Wildlife Parasites II

A30

Screening of gastrointestinal parasites of dogs from municipal kennels in Portugal

P. Lopes[1], J. Gomes[2,3,4], J. Lozano[3,4], M. Louro[3,4], L. Madeira de Carvalho[3,4], I. Pereira da Fonseca[3,4], M. V. Cunha[1,5]

[1]Centre for Ecology, Evolution and Environmental Changes (cE3c) & Associate Laboratory CHANGE, Lisboa, Portugal
[2]Agrarian School of Elvas, Polytechnic Institute of Portalegre, Department of Agrarian and Veterinary Sciences, Portalegre, Portugal
[3]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal
[4]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisboa, Portugal
[5]Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

Introduction: Gastrointestinal parasites (GIP) are frequently reported in dogs worldwide, some of which are potentially zoonotic. Stray and shelter dogs are more prone to carry and spread GIP, but their prophylactic or therapeutical control is generally unknown. In Portugal, municipal kennels are facilities that shelter stray animals, but routine coprological analyses are rarely performed upon animal admission. **Objectives:** We performed a nationwide screening of GIP in dogs at municipal kennels and investigated the underlying potential causal factors. **Materials & Methods:** Between November 2021-October 2022, 355 dog stool samples were collected in several municipal kennels in Portugal, and then processed with the Mini-FLOTAC technique, using zinc sulphate flotation solution. The influence of animal- and kennel-related variables in parasite presence was assessed by Fisher's exact tests, with 95% significance. **Results:** Approximately 17% (59/355) of the samples were GIP-positive. *Trichuris* sp. (20/355), Toxocaridae (19/355), Ancylostomatidae (13/355), and *Cystoisospora* sp. (8/355) were the most frequently observed parasites. Most of the positive samples (53/59) had more than 10 eggs or oocysts per gram. The results revealed that the region and number of dogs housed in kennels were significantly associated with the presence of Ancylostomatidae ($p< 0.001$ and $p=0.013$, respectively) and *Trichuris* sp. ($p=0.04$ and $p=0.03$, respectively). Also, season was significantly associated with the presence of Ancylostomatidae eggs ($p=0.004$), while stool consistency and dogs' age range were significantly associated with Toxocaridae eggs ($p=0.008$ and $p=0.049$, respectively). **Conclusion:** To the best of our knowledge, this is the first nationwide screening of GIP in shelter dogs. The low overall GIP prevalence suggests that the internal deworming protocols in the assessed kennels are generally effective, with all of them deworming dogs internally upon admission, contributing to explain these results. However, our study also highlights the importance of considering several factors when establishing internal deworming protocols, including the intervention region encompassed by the kennel, the number of dogs housed and their age. **Funding:** Strategic Funding from FCT to cE3c (UIDB/00329/2020), CHANGE (LA/P/0121/2020), CIISA/FMV (UIDB/00276/2020), AL4AnimalS (LA/P/0059/2020), and to PL (2021.09447.BD), JL (2020.09037.BD) and ML (UI/BD/152818/2022), is gratefully acknowledged.

A31

Heavy infection with *Contracaecum rudolphii* A larvae in the intestine of *Anguilla anguilla*: Pathobiology and inflammatory response

B. Sayyaf Dezfuli[1], *L. Giari*[2], *G. Castaldelli*[2], *M. Lanzoni*[2], *G. Bosi*[3]

[1]University of Ferrara, Life Sciences & Biotecnology, Ferrara, Italy
[2]University of Ferrara, Department of Environmental and Prevention Sciences, Ferrara, Italy
[3]Università di Milano, Health, Animal Science and Food Safety, Milan, Italy

Introduction

The European eel, *Anguilla anguilla*, is a major warm water fish species and *Contracaecum rudolphii* uses eel and several other fish species as paratenic host. Fish response to extra-intestinal parasites induce the formation of granuloma and granulomas are chronic inflammatory lesions that appear as nodules in one or more organs. Larvae (L3) of *C. rudolphii* A were encapsulated within the thickness of the eel intestinal wall and within a granuloma on the external visceral peritoneum (serosa). Information on the status of the eel immune system is of importance to maintain good health throughout the grow out period in eel fisheries.

Objectives

There is limited knowledge in terms of both cellular and humoral immune responses of *A. anguilla* to helminths. Identification of the host immune cells only with histology appeared to be insufficient. The current investigation was undertaken to gain information on the presence and distribution of 5 antibodies in different types of immune cells of *A. anguilla* intestine against *C. rudolphii* A (L3) larvae.

Materials & Methods

From January to October 2022, a subsample of 40 eels from the Comacchio lagoons were examined, several 15 x 15 mm pieces (from infected-uninfected intestine) were excised and fixed in 10% neutral buffered formalin for 24 h and were assessed by histology techniques. For immunohistochemistry following molecules were tested: antibodies against immunoglobulin E (IgE)-like receptor (FCεRIγ), histamine, serotonin, tryptase and inducible-nitric oxide synthase (i-NOS).

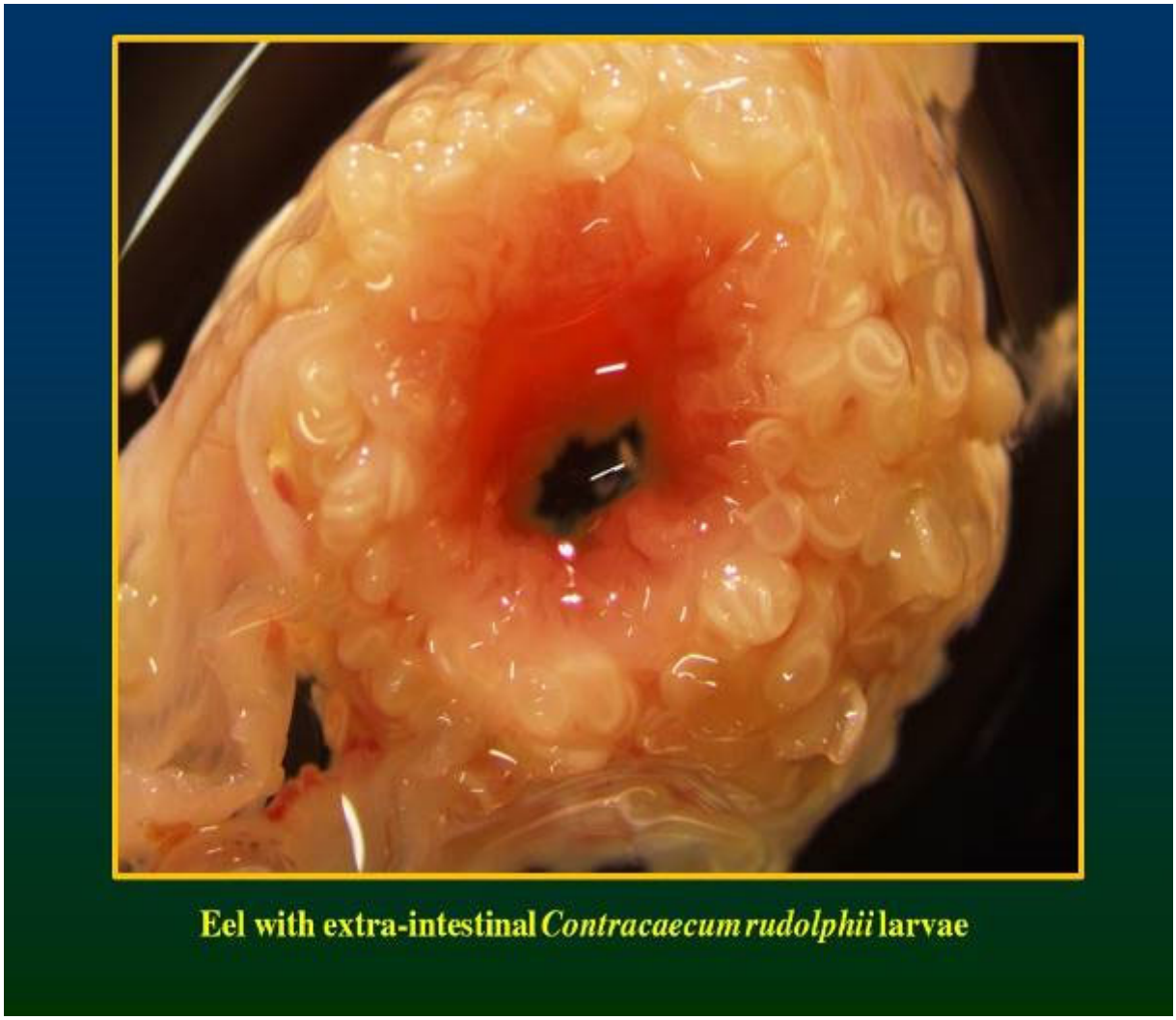
Results

Twenty-four eels (60%) out of 40 harboured larvae (L3) of the nematode *C. rudolphii* A, the intensity of infection ranged from 5 to 76 parasites per eel. Conspicuous granulomas were visible on outer surface of the intestine. The outer part of the granuloma contained macrophages, macrophage aggregates, and mast cells (MCs) scattered among fibroblasts. The middle layer was rich in MCs and fibroblasts and the inner layer consisted mainly of dark epithelioid cells. Within the granulomas cells of different types were positive to antibodies anti-FCεRIγ, -histamine, -serotonin, -tryptase and -i-NOS.

Conclusion

Immune evasive mechanisms render the parasites able to survive even years in tissue despite the high abundance of host immune cells in vicinity. This survey might suggest an evolutionary trade-off between eel tolerance and relatively inactive nematode larva within granuloma.

Fig. 1



A32

Evaluating the *in vivo* efficacy of using the predatory fungus *Mucor circinelloides* (FMV-FR1) in the reduction of coccidia shedding in a peacock collection

J. Lozano[1,2], C. Almeida[3], E. Vicente[4], D. Sebastião[4], M. Oliveira[1,2], A. Paz-Silva[5], L. Madeira de Carvalho[1,2]

[1]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal
[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisboa, Portugal
[3]Exoclinic – Clínica Veterinária de Aves e Exóticos, Lisboa, Portugal
[4]Castelo de São Jorge, EGEAC – Empresa de Gestão de Equipamentos e Animação Cultural, Lisboa, Portugal
[5]Control of Parasites Research Group (COPAR, GI-2120), Department of Animal Pathology, Faculty of Veterinary, University of Santiago de Compostela, Lugo, Spain

Introduction: Since the late 1990"s, it has been demonstrated that the use of predatory fungi is a sustainable approach for the integrated control of gastrointestinal (GI) parasites affecting domestic, exotic and wild captive animals. However, studies performed in birds are still scarce.

Objectives: This pioneer research aimed to assess the *in vivo* efficacy of using the ovicidal fungus *M. circinelloides* for reducing coccidia shedding in a peacock collection.

Materials & methods: This trial was performed between October and December 2022, in the resident peacock collection (n=60 birds) of São Jorge Castle, at Lisbon, Portugal. A total of 33 fungal administrations were performed, P.O. and 3x/week, using supplements composed by 600 g of bird feed mixed with 60 mL of a *M. circinelloides* (FMV-FR1) suspension (10^6 spores/bird). Feces were collected every 15 days, and then processed and analysed using the Mini-FLOTAC technique, to calculate the coccidia shedding (Oocysts per Gram of Feces – OPG). This bird collection served simultaneously as control (t0 days) and test group (t15-t90 days). Parasite shedding for each test timeframe was compared with control, using the Mann-Whitney Test, while the appearance of samples (normal vs blood and/or diarrhoea) was assessed using the Fisher Exact Test (95% significancy).

Results: Peacocks were initially positive for *Eimeria* sp. (20107 ± 8034 OPG), and 88% (15/17) of the samples had normal appearance. Afterwards, it was possible to observe an overall decrease in the coccidia shedding, with reduction efficacies of 62%, 78%, 78%, 92%, 70% e 59%, after 15, 30, 45, 60, 75 and 90 days of fungal feeding, respectively, and with the values for days 45 and 60 being significant (p=0.004 and p=0.012, respectively). Finally, feces appearance did not differ between each test timeframe and the control time point.

Conclusion: This strain of *M. circinelloides* was effective in reducing coccidia parasitism in peacocks, being the first study of this kind in birds, worldwide. Results suggest that this fungal strain might be used in integrated programs for the GI parasite control.

Funding: This research was funded by CIISA/FMV Project UIDB/00276/2020 and LA/P/0059/2020 - AL4AnimalS (both funded by FCT). Also, João Lozano owns a PhD research fellowship 2020.09037.BD (funded by FCT).

A33

Echinococcus spp. Namibian wildlife

O. Aschenborn[1,2,3], P. Beytell[4], J. Melzheimer[2], U. Mackenstedt[1], T. Romig[1], M. Wassermann[1]

[1]University of Hohenheim, Parasitology, Stuttgart, Germany
[2]Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
[3]University of Namibia, School of Veterinary Medicine, Windhoek, Namibia
[4]Ministry of Environment and Tourism, Directorate of Scientific Service, Windhoek, Namibia

Introduction: Cystic Echinococcosis is a worldwide occurring zoonotic disease and can be caused by five distinct species of the cestode genus *Echinococcus*. Only in Africa are all five species present. Whereas *E. felidis* was so far only be found in wildlife, the lifecycle of the four other species, *E. granulosus* sensu stricto (s.s.), *E. ortleppi*, *E. canadensis* (G6/7) and *E. equinus*, is clearly associated with various livestock species and domestic dogs. However, previous studies showed that some of those domestic *Echinococcus* species can also be found in wild animals, e.g. *E. granulosus* s.s. in Kenyan lions. There is, however, little known about the extent to which wild animals are involved in the different lifecycles and their maintenance.

Objective: The present study was conducted to learn more about the situation of *Echinococcus* spp. in wildlife in Namibia.

Material & Methods: At various locations in Namibia, faecal samples were collected from 224 wild carnivores and examined for the presence of taeniid eggs, and 300 possible wild intermediate hosts were examined for the presence of cysts. For species identification, eggs and cysts were analysed by PCR and sequencing of the *nad1* or *cox1* gene.

Results: *Echinococcus* spp. could be identified in 31 carnivorous and 39 intermediate hosts. As anticipated, the wildlife adapted *E. felidis* could be detected in lions (7) and warthogs (4). However, the livestock associated species were also present. *Echinococcus granulosus* s.s. was found in African wild dog (2), *E. ortleppi* in black-backed jackals (2) and oryx antelopes (6) and *E. equinus* in lions (9), black-backed jackals (2) and zebras (27). The *Echinococcus* species detected in the most wildlife species was *E. canadensis* G6/7, namely in lions (3), cheetahs (3), African wild dogs (1), black-backed jackals (2) and oryx antelopes (2).

Conclusion: All five *Echinococcus* species have been detected in Namibian wildlife, although only *E. ortleppi*and *E. canadensis* G6/7 have been found so far in Namibian cattle and sheep, respectively. These results indicate that the presence of *Echinococcus* spp. in wildlife is not accidental, but wild animals are important links in the parasitic lifecycle, at lease in the study area.

A34

Seal louse and heartworm: Time trends and impact on harbour seals in the North and Baltic Sea

I. Herzog[1], A. Preuss[2], P. Wohlsein[3], U. Siebert[1], S. N. Gorb[2], K. Lehnert[1]

[1]Tierärztliche Hochschule Hannover, Institute for Terrestrial and Aquatic Wildlife Reseach, Büsum, Germany
[2]Zoological Institute of the university of Kiel, Department of Functional Morphology and Biomechanics, Kiel, Germany
[3]Tierärztliche Hochschule Hannover, Department of Pathology, Hannover, Germany

Pinnipeds provide a livelihood for numerous parasites, showing unique morphological and physical adaptions to the semiaquatic lifestyle of their host. *Echinophthirius* (*E.*) *horridus*, the seal louse and the heartworm *Acanthocheilonema* (*A.*) *spirocauda* outline a possible unique vector-parasite congregate transported to the marine environment. Commonly mild health effects are reported, yet they can cause anaemia, alopecia or arteritis and obstruction of vessels in severe cases. *E.horridus* is discussed to play an obligatory role as vector for heartworm filariae (*A. spirocauda*) of harbour seals (*Phoca vitulina*). In this study we examined the prevalence of *A.spirocauda* and *E.horridus* in harbour seals (n = 672) between 2014 and 2021 to analyse time trends and life history traits using a unique sample set collected within a stranding network along the North and Baltic Sea coast comprising necropsy results, histological data and tissue archive. Additional Confocal Laser Scanning Microscopy (CLSM), nanoCT and attachment force measurements were performed to characterise physical solutions for life on marine hosts.Infection of harbour seals with *A.spirocauda* was found in 11% (n = 76). The prevalence of *A.spirocauda* varied over the seven-year-study period, and showed a notable increase compared to previous decade. Seal lice were found in 4% (n = 26) of the examined harbour seals. Seal lice prevalence remained relatively low. For both parasites, there were no sex related differences in infection patterns but a higher prevalence in yearling and young–of-the-year seals. Histological screening of seal lice revealed larval nematode stages in the hemocoel supporting the hypothesis of seal lice functioning as intermediate host and vector of *A.spirocauda*. Additional histology showed lesions affecting the health status of their host. CLSM of *E.horridus* indicates sclerotized structures and nanoCT aided in 3D reconstruction of musculoskeletal system, providing first understandings of attachment mechanisms. Increased prevalence of *A.spirocauda* over the study period may reflect growing seal populations, higher densities on haul-outs, thereby facilitating transmission by lice vectors. Higher prevalence in younger age groups might reflect the social behaviour of harbour seals within the different age groups; it could also indicate a vertical transmission pattern. The results underline, parasites are useful indicators for host ecology and ecosystem changes in the North and Baltic Sea.

A35

Plasmodium and beyond - haemosporidian parasites of a Malagasy bird population

S. Musa[1], F. Woog[2], A. Dinkel[1], U. Mackenstedt[1]

[1]Univerity of Hohenheim, Parasitology, Stuttgart, Germany
[2]State Museum of Natural History Stuttgart, Stuttgart, Germany

Haemosporida are important intracellular protozoan parasites. In addition to the genus *Plasmodium*, other genera also belong to this order: *Haemoproteus* and *Leucocytozoon*, among others. Whereas species of the order *Plasmodium* can be found in all vertebrate taxa, *Haemoproteus* and *Leucocytozoon* are specialized on birds. Basic knowledge of prevalence, diversity and impact of all avian haemosporidian parasites is still scarce. A long-term, large-scale molecular study of a bird population in Madagascar is providing new insights into the parasite population that exists there. An enormous number of unknown haemosporidian lineages were found, showing that Madagascar is not only a biodiversity hotspot for birds, but also for their parasites. Each haemosporidian parasite differs in its degree of host specialization and abundance. Understanding those parasite-host interactions is crucial to assess the impact of these parasites on the environment.

Fig. 1

Session VI – Sex and Gender in Parasitology

A36

Sex difference in hepatic amebiasis

H. Lotter[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Molecular infection Immunology, Hamburg, Germany

Infections with the protozoan parasite *Entamoeba (E.) histolytica* can lead to hepatic amebiasis, a severe, focal liver injury that is more common in men than in women. In the first and second funding period, we investigated immune mechanisms underlying liver damage and regeneration during intrahepatic parasitic infection the murine model for the disease. In summary, we found that initiation of the IL-23/IL-17 immunopathological axis leads to massive recruitment of classical, pro-inflammatory Ly6Chi monocytes through induction of C-C chemokine ligand 2 (CCL2). Liver destruction was finally mediated by TNFa produced by monocytes as well as liver resident macrophages. On the other hand, regeneration was promoted by anti-inflammatory, IL13+Arg1+ Ly6Clo monocytes. In the last funding period, we questioned whether i) sex hormones modulate the immunopathological functions of pro-inflammatory monocytes in the murine model for the disease and whether this is transferable to their human correlates, ii) which role plays the Hypoxia-inducible factor-1 alpha (HIF-1a) in balancing liver damage in male and female individuals and iii) what are the characteristics for immunopathological and protective monocytes. Hepatic amebiasis occurs with a clear sex bias towards male individuals. We have previously shown, that testosterone was found to have important functions for the development of hepatic amebiasis in mice, but a link between testosterone and monocytes has not been identified so far. *In vivo* as well as *in vitro* studies now revealed, that testosterone treatment triggers proinflammatory responses in human and murine classical monocytes. Testosterone substitution of castrated male mice increases the frequency of TNFa, CCL2 and CXCL1-producing classical monocytes during hepatic amebiasis, the latter cytokine further amplifies the recruitment of Ly6Chi monocytes but also that of neutrophils, which also contribute to tissue damage in hepatic amebiasis. Furthermore, androgen treatment of isolated human monocytes but also of individuals, undergoing gender reassignment, increased the production of these cytokines, supporting the hypothesis that androgens may contribute to an increased risk of developing monocyte-mediated pathologies.

A37

Kinetic analysis of IL-9 expressing cells during murine infection with the parasitic nematode *Strongyloides ratti*

W. Hartmann[1], L. Heepmann[1], L. Linnemann[1], P. Lincona Limon[2], M. Breloer[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]Instituto de Fisiologia Celular, Mexico City, Mexico

Introduction: During their migration helminths induce the release of tissue-derived alarmins such as IL-33 which promote the initiation of a protective type 2 immune response. We have previously demonstrated that mucosal mast cells are central for the timely ejection of *Strongyloides ratti* from the intestine of Infected mice. Application of recombinant IL-33 or enhancement of endogenous IL-33 accelerated the degranulation of mucosal mast cell in an IL-9 dependent manner, resulting in rapid expulsion of the *S. ratti*.

Objective: Aim of the study was to identify the cellular sources of IL-9 in *S. ratti* infected mice.

Material and Methods: BALB/c IL-9 GFP Reporter mice were used to analyze IL-9 expressing cells in 4 different groups: naïve, IL-33 treated, IL-33 treated + *S. ratti* infected and *S. ratti* infected mice. Lung, mesenteric lymph nodes (mLN) and lamina propria-derived cells were isolated at day 2, 6 and 10 p.i. Frequencies of granulocytes, mast cells, B cells, NK cells, various T cell (CD4, CD8, gd T cells) and innate lymphoid cell (ILC) subsets were analyzed in several organs and their expression of the pluripotent cytokine IL-9 was quantified by flow cytometry.

Results: Kinetic analysis revealed a pronounced expansion and expression of IL-9 GFP by ILC subsets such as ILC2 and NK cells in lung and mLN cells of IL-33 treated mice, as expected. ILC2s were identified as the main IL-9 expressing cell type in the lung of all 3 groups compared to naïve mice. Strikingly, the expansion of these cell types was not visible in the lung and mLN of *S. ratti* infected mice and was not altered by additional IL-33 treatment. In the small intestine (lamina propria) we observed a late expression of IL-9 by GATA-3$^+$ CD4$^+$ T cells and mast cells at day 10 p.i. in *S. ratti* infected mice. Expression of IL-9 by these cells was already detectable at day 6 p.i. in IL-33 treated + *S. ratti* infected mice and was absent in IL-33 treated mice indicating a *S. ratti*-specific expression of IL-9 at the site of infection.

Conclusion: Artificial activation of innate cells by application of the alarmin cytokine IL-33 induces a rapid and pronounced expression of IL-9 by ILC subsets. By contrast, simultaneous infection with *S. ratti* dampened the expansion of ILC subsets indicating counter regulation of IL-33 induced anti-helminth immune response.

A38

The role of CD160 in the control of helminth infection

L. Heepmann[1], W. Hartmann[1], L. Linnemann[1], L. Scherer[1], M. Breloer[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Helminth Immunology, Hamburg, Germany

Introduction:

Helminths dampen their host`s immune response. Among other things, helminths induce expansion of regulatory lymphocytes and checkpoint molecules with co-inhibitory functions such as B and T lymphocyte attenuator (BTLA), a ligand of Herpes Virus Entry Mediator (HVEM). Another HVEM ligand is CD160, which is described on various T cell subsets, NK cells, innate lymphoid cells (ILCs) and mast cells.

Objective:

Aim of the study was to investigate the role of the CD160 in helminth infected mice.

Material and Methods:

I used *Strongyloides ratti* was used as a model for helminth infection with a tissue migration and an intestinal phase. Taking advantage of CD160-deficient ($CD160^{-/-}$), we performed a tight kinetic study of *S. ratti* infection. Furthermore, the impact of CD160 on the innate immune response using $RAG^{-/-}$mice that lack T and B lymphocytes but express CD160, and $CD160^{-/-}RAG^{-/-}$ mice that additionally lack CD160 was investigated. Worm burden was analyzed at different timepoints. Flow cytometry was used to analyze which cell types express CD160 in *S. ratti*-infected mice. Additionally the expansion/contraction of CD160-expressing cells during *S. ratti* infection was investigated.

Results:

Numbers of migrating larvae in the tissues were unchanged but numbers of adult worms in the intestine day 6 p.i. were reduced in $CD160^{-/-}$ mice compared to wildtype mice. $CD160^{-/-}RAG^{-/-}$mice also showed reduced intestinal parasite burden day 6 p.i. Although $RAG^{-/-}$ mice cannot completely eject *Strongyloides* parasites, they control the parasite burden from initially ca 100 parasites to 2-5 parasitic adults per mouse. In RAG-/- mice with additional absence of CD160the initially lower parasite burden day 6 p.i. remained stable for almost 100 days. This was associated with impaired mast cell activation in $CD160^{-/-}RAG^{-/-}$mice.

Intestinal NK cells, ILC1, ILC2 and ILC3 express CD160 in naïve mice. During *S. ratti* infection sSpecifically, $CD160^{+}$ ILC2 expand while CD160-deficiency interfered with ILC2 but not with NK cell expansion during infection.

Conclusion:

In combination, these results suggest that CD160-mediated signals have dual and opposing functions during intestinal helminth infection, antagonizing early innate immune responses but promoting late innate immune response.

A39

Characterization of the regulative role of the C-type lectin receptor MINCLE in the initiation of anti-helminth immune responses

L. Linnemann[1], J. Antwi[1], V. Gnanapragassam[2], T. Ramcke[1], B. Lepenies[2], M. Breloer[1]

[1]Bernhard-Nocht-Institut für Tropenmedizin, Helminth Immunology, Hamburg, Germany
[2]Stiftung Tierärztliche Hochschule Hannover, Inst. für Immunologie, Hannover, Germany

Objectives

One quarter of the human population is infected with helminths, large multicellular parasites, that are controlled in the context of a type 2 immune response. Using Strongyloides ratti infection in mice, we aim to analyse the role of C-type lectin receptors (CLR), an ancient family of innate pattern recognition receptors, in the initiation and modulation of protective type 2 immunity to helminths.

Methods

Screening a CLR-hFc library, we found yet unidentified S. ratti derived ligand(s), engaging Macrophage inhibitory C type lectin receptor (MINCLE) and further signalling into a MINCLE reporter cell line. We used a MINCLE-deficient mouse to analyse this interaction and its effects in vitro and in vivo.

Results & Conclusions

Strikingly, the loss of this CLR in MINCLE-deficient mice resulted in reduced intestinal parasite burden when compared to wildtype mice, suggesting absence of the CLR did not impair but improved the host defence immune response. Parameters of Type 2 immunity like mucosal mast cell activation, S. ratti specific Th2 cell responses, protective memory and also the intestinal microbiota were unchanged in MINCLE-deficient mice. However, an increase in MINCLE+ cell populations, like eosinophils, neutrophils and dendritic cells, was observed in the lungs and intestine, offering a possible target for a MINCLE-ligand. Additionally, in vitro stimulation of MINCLE+, but not MINCLE-deficient, macrophages and dendritic cells with a baseline concentration of the MINCLE agonist TDB and increasing concentrations of S. ratti lysate, lead to a dose dependent reduction of TDB-induced TNFα production. By contrast, production of TDB-induced IL-6 and IL-10 were synergistically increased by co-stimulation with S. ratti lysate. We are currently investigating the nature of the putative S. ratti -derived MINCLE ligand and its modulatory effects on the hosts immune response.

A40

Microfilariae-induced eosinophil ETosis is NADPH oxidase- and inflammasome-dependent

A. Ehrens[1,2], C. Nieto Perez[1], A. Krez[1], F. Risch[1], B. Lenz[1], N. Offermann[3], M. Koschel[1], M. Capasso[3], A. Hoerauf[1,2], M. P. Hübner[1,2]

[1]University Hospital Bonn, Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany
[2]German Center for Infection Research (DZIF), Partner site, Cologne/Bonn , Bonn, Germany
[3]German Center for Neurodegenerative Diseases (DZNE) within the Helmholtz Association, Immunregulation, Bonn, Germany

Introduction

Eosinophils as well as their cytotoxic granules are important for protective immunity against parasitic filarial nematodes, which can be mediated through extracellular DNA trap cell death (ETosis). Thereby, intracellular DNA is explosively released, entrapping and killing pathogens. Neutrophil ETosis (NETosis) can be induced in a NADPH oxidase (NOX) or Ca^{2+}-dependent manner. Moreover, the activation of the inflammasome can lead to NETosis with gasdermin D (GSDMD) being an essential enzyme in forming pores and thus, releasing the DNA traps. However, eosinophil ETosis (EETosis) is understudied and the exact signaling cascade for filaria-induced ETosis is not known yet.

Objectives

The current study investigated the signaling pathway during EETosis in response to microfilariae (MF) using the rodent filaria *Litomosoides sigmodontis*.

Materials & Methods

To study EETosis in response to MF, eosinophils were generated from the bone marrow of wild-type and different knockout mice (NOX, caspase-1, ASC, NLRP3 and AIM2) and cells were stimulated with MF. EETosis was investigated using fluorescence microscopy as well as DNA quantification methods.

Results

Our results demonstrate that MF induce EETosis in a NOX-dependent manner since eosinophils generated from NOX knockout mice did not release DNA traps in response to MF and PMA. In addition, similar to PMA, histone citrullination occured during a later state of MF-induced EETosis. Moreover, the canonical inflammasome pathway is involved during EETosis. The canonical inflammasome assembles after activation of a sensor molecule, which recruits pro-caspase-1 and the adaptor molecule ASC. As an executing caspase, caspase-1 is able to cleave GSDMD, which forms pores in the cellular membrane. During MF-induced EETosis, active caspase-1 was detected in DNA traps. Inhibition of caspase-1 as well as eosinophils generated from caspase-1 or ASC knockout mice failed to release DNA in response to MF. Using eosinophils from AIM2 and NLRP3 knockout mice, we identified AIM2 and not NLRP3 as the responsible inflammasome sensor, since AIM2 knockout eosinophils, in contrast to NLRP3 knockout eosinophils, were unable to form DNA traps in response to MF. Moreover, we visualized GSDMD during EETosis and observed pore formation in the membrane and the nucleus.

Conclusion

Our results indicate that EETosis in response to microfilariae is dependent on NOX and the AIM2-caspase 1 inflammasome.

A41

Repeated sensitization of mice with microfilariae of *Litomosoides sigmodontis* induce pulmonary eosinophilia in an IL-33 dependent manner

B. Lenz[1], A. Ehrens[1,2], J. Gal[3], J. Ajendra[1], F. Risch[1], H. McSorley[4], C. Martin[3], A. Hoerauf[1,2], M. P. Hübner[1,2]

[1]University Hospital Bonn, Institute for Medical Microbiology and Parasitology, Bonn, Germany
[2]German Center for Infection Research (DZIF), Partner site, Cologne/Bonn , Bonn, Germany
[3]Muséum National d'Histoire Naturelle, Unité Molécules de Communication et Adaptation des Microorganismes, Paris, France
[4]University of Dundee, Cell Signalling and Immunology, Dundee, United Kingdom

Background: Eosinophilia is a hallmark of helminth infection and eosinophils are essentially involved in the protective immune responses against helminths. The distinct role of eosinophils during parasitic filarial infection, allergy and autoimmune disease-derived pathology is still not sufficiently understood. In this study, we established a mouse model for tropical pulmonary eosinophilia (TPE), a clinical manifestation that is caused by eosinophil hyper-responsiveness within the lung of lymphatic filariasis patients to investigate involved mechanisms.

Methods: Wild-type (WT) BALB/c mice were sensitized with dead microfilariae (MF) of the rodent filarial nematode *Litomosoides sigmodontis* in weekly intervals for a total of three times and subsequently challenged with viable microfilariae (MF) to induce TPE. The resulting immune response was compared to non-sensitized wild-type mice as well as sensitized eosinophil-deficient dblGATA mice using flow cytometry, lung histology and ELISA. Further, the role of IL-33 signaling during TPE development was investigated using the IL-33 signaling blocker HpARI.

Results: Sensitized WT mice displayed increased eosinophil numbers in blood, BAL, spleen and lung. The eosinophilia was accompanied with enhanced MF clearance from the blood, increased immune cell numbers in the lung, increased IL-33 serum levels and activation of lung eosinophils in TPE-induced WT mice in comparison to non-sensitized WT mice and dblGATA TPE mice. Further, TPE-induced WT mice had an increased number of group 2 innate lymphoid cells (ILC2) and alternatively activated macrophages (AAM) within the lung when compared to non-sensitized WT mice. Lung retention of MF was drastically increased in sensitized mice. Interestingly, blocking of IL-33 signaling prevented a type–2 immune shift, reducing M2 macrophages, lung ILC2s and eosinophils.

Conclusion: Repeated sensitization with *L. sigmodontis* MF induces pulmonary eosinophilia accompanied with a strong type 2 immune response mimicking human TPE. Using this newly established *L. sigmodontis* TPE mouse model, we demonstrate that IL-33 is a key alarmin, which is essentially involved in the induction of TPE-associated immune responses.

A42

Lung immune responses during infection with the filarial nematode *Litomosoides sigmodontis* – crosstalk between lung and pleural cavity

J. Ajendra[1,2], C. Guth[1], P. Schumacher[1], B. Lenz[1], B. Chan[2], J. E. Parkinson[2], A. Hoerauf[1,3], J. E. Allen[2], M. P. Hübner[1,3]

[1]University Hospital Bonn, Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany
[2]The University of Manchester, Lydia Becker Institute for Infection and Inflammation, Manchester, United Kingdom
[3]German Center for Infection Research (DZIF), Partner site, Cologne/Bonn , Bonn, Germany

Introduction

Recent advances in immunological studies have reinforced the existence of communication between tissues and organs, contributing to maintenance of host homeostasis but also to disease development. Helminth parasites are a great tool to investigate such inter-tissue communication because of their migration through different tissues and organs within their hosts.

Objectives

The filarial nematode *Litomosoides sigmodontis* (*Ls*) resides in the pleural cavity but has a transient lung stage between days 3-5 post infection and later during patency, microfilariae (MF) can accumulate in the lungs. However, pulmonary immune response during *Ls* infection are not well characterized so far. Here, we present a detailed overview of the ongoing immunological events within the lungs over the course of *Ls* infection. Further, we aimed to investigate whether immune responses within the lung can affect responses in the pleural cavity, the site of infection.

Materials & Methods

We used multi-color flow cytometry to investigate cellular composition, T cell and macrophage phenotypes as well as neutrophil-eosinophil kinetics in the lung and compared them side-by-side with the responses within the pleural cavity. ELISA and qPCR were performed to investigate cytokine/chemokine milieu and type 2 associated genes to obtain an overview of ongoing immune responses.

Results

We analyzed the early events (d3-5pi) of the lung response and found that BALB/c mice, but not semi-susceptible C57BL/6 mice that clear the infection shortly after the molt into adult filariae, upregulate IL-17A production by γδ T cells. This was associated with increased early lung tissue damage only seen in BALB/c mice. Depleting this early lung IL-17A resulted in significantly lower CD4+ T cell and eosinophil numbers and decreased production of the type 2 cytokines IL-5 and IL-13 in the pleural cavity d15pi. Currently we are deciphering the mechanisms by which pulmonary IL-17A regulates the pleural cavity response. Furthermore, with onset of microfilaremia, the lung displays an immune reaction similar to the pleural cavity, even though L4 and adult stages solely live in the pleural cavity. Interestingly, MF+ mice have a higher eosinophil frequency in the lung compared to MF- mice, indicating MF-dependent immune responses.

Conclusion

Our data suggests a crosstalk between the lung and the pleural cavity that supports the induction and maintenance of protective immune responses against filariae.

A43

A comparative proteomic analysis of *Toxoplasma gondii* RH wild-type and four SRS29B (SAG1) knock-out clones to understand the impact of *sag1* disruption on the gene expression profile during the lytic cycle *in vitro*

K. Hänggeli[1], A. Hemphill[1], N. Müller[1], J. Müller[1], M. Heller[2], G. Boubaker[1]

[1]Institut für Parasitologie der Universität Bern, Bern, Switzerland
[2]Proteomics and Mass Spectrometry Core Facility, Department for Bio Medical Research, Bern, Switzerland

The role of the major *T. gondii* tachyzoite surface antigen 1 (SAG1), has remained elusive. We have previously generated four *T. gondii* RH SAG1 knock-out (KO) strains by CRISPR-Cas9 and characterized these KOs regarding the number of integrated *dihydrofolate reductase-thymidylate synthase* (*mdhfr-ts*) drug selectable marker. Accordingly, two clones have a single integration of the *mdhfr-ts* into the SAG1 gene. In one clone, *sag1* was disrupted by insertion of a short DNA sequence unrelated to the resistance marker, but *mdhfr-ts* was integrated elsewhere in the genome. Another clone exhibited an insertion of *mdhfr-ts* into *sag1*, but several other *mdhfr-ts* copies were found in the genome.

In this study, we determined the overall change(s) in protein expression patterns in all four clones by applying a comparative shotgun proteomics approach in relation to wild-type (WT) parasites using biological triplicates of each KO and WT clone.

In SAG1 KO strains, the expression levels of 53 proteins were significantly altered. Of these, 12 were upregulated (fold change (FC) ≥ 1.5) and 41 were downregulated (FC ≤ 0.66) as compared to the WT parasites. In KO parasites, the expression of peptidase family M3 protein and putative transmembrane protein were 8- and 7-fold upregulated, respectively, and the expression of CBS domain-containing protein and TBC domain-containing protein was reduced by 90%, compared to WT tachyzoites. 39 SAG1 related sequence (SRS) proteins were found to be expressed in WT tachyzoites, and among those five were upregulated and three were downregulated in SAG1 KO parasites. In addition, depletion of SAG1 expression also impacted dense granule (GRA) protein expression; GRA 9 and GRA2 were upregulated. With regard to the phenotype, scanning electron micrographs did not reveal notable differences in the shape or dimensions of tachyzoites, but the surface of the mutants lacking SAG1 exhibited a higher density of particles compared to the smoother surface of WT tachyzoites. Plaque assays revealed reduced growth of KO tachyzoites compared to WT parasites.

In conclusion, this comprehensive analysis shows, for the first time, that the lack of SAG1 expression has a profound impact on the tachyzoite proteome, including the tachyzoite surface composition, and affects the tachyzoite growth characteristics *in vitro*. This highlights the need for further *in vivo* studies to elucidate the functional role of SAG1 during infection in the mammalian host.

A44

Functional repurposing of anionic phospholipids for regulating calcium homeostasis in *Toxoplasma gondii*

D. A. Katelas[1]

[1]Humboldt University of Berlin, Molecular Parasitology , Berlin, Germany

Dimitrios Alexandros Katelas, Rosalba Cruz Miron, Nishith Gupta

Department of Molecular Parasitology, Faculty of Life Sciences, Humboldt University, Berlin, Germany

Introduction

Toxoplasma gondii is a prominent apicomplexan parasite infecting many warm-blood organisms. Its lytic cycle in human host cells requires a range of phospholipids, some of which have emerged as key players in signaling and calcium regulation.

Objectives

Following our previous research, this work aimed to evaluate the synthesis and roles of anionic phospholipids during the lytic cycle of *T. gondii*.

Materials & Methods

We engineered a repertoire of parasite mutants (knockout, conditional knockdown, and overexpression strains) and phenotyped them by standard molecular biology methods, including immunofluorescence, western blot, and lipidomic assays.

Results

Herein, we show that tachyzoites of *T. gondii* harbor two similar enzymes in the endoplasmic reticulum, catalyzing the synthesis of phosphatidylserine (PtdSer) and phosphatidylthreonine (PtdThr). Transgenic expression of a gene-encoded biosensor (LactC2-GFP) revealed the distribution of two phospholipids in the parasite ER and inner membrane complex. While tachyzoites could survive deletion of PtdThr synthase (PTS), *de novo* synthesis of PtdSer is essential for the lytic cycle. Notably, the PTS mutant displayed a defective calcium homeostasis, resulting in impaired gliding motility and concomitant invasion and egress events. Our lipid binding assays disclosed interaction of PtdThr and PtdSer with the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump, and its ectopic overexpression in the PTS mutant restored its lytic cycle. Conversely, auxin-mediated knockdown of SERCA and PtdSer synthase abrogated the parasite development with evident defects in replication and locomotion. Our independent work on P4-ATPases (Chen et al, JBC 2021; CSBJ, *in review*) has discovered a PtdSer/PtdThr flippase located at the apical end of tachyzoites that contributes to the motility-dependent invasion and egress.

Conclusion

PtdSer in mammalian cells is known to regulate the calcium release *via* IP_3 channel, which remains to be identified in *T. gondii* (*ongoing work*). These data, taken together, strongly suggest evolutionary speciation and functional repurposing of two analogous glycerophospholipids to cater the lytic cycle of *T. gondii*.

A45

Whole genome sequences of isolates across Europe reveal more diversity within European *Toxoplasma gondii* type II than expected

P. Maksimov[1], P. Angulo Lara[2], L. Galal[3], R. Calero-Bernal[2], M. Fernández-Escobar[2], A. Mercier[3], H. Lorenzi[4,5], M. Joeres[1], B. Koudela[6,7], R. Blaga[8], M. Globokar Vrhovec[9], K. Stollberg[10], N. Bier[10], S. Sotiraki[11], J. Sroka[12], W. Piotrowska[12], P. Kodym[13], W. Basso[14], F. J. Conraths[1], M. L. Dardé[3], F. Spano[15], P. Vatta[15], S. Caccio[15], P. Jokelainen[16], L. M. Ortega-Mora[2], G. Schares[1]

[1]Friedrich-Loeffler-Institut, Institute of Epidemiology, Greifswald, Germany
[2]SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid, Madrid, Spain
[3]Inserm U1094, IRD U270, Univ. Limoges, CHU Limoges, EpiMaCT - Epidemiology of chronic diseases in tropical zone, Institute of Epidemiology and Tropical Neurology, OmegaHealth, Limoges, France
[4]The J. Craig Venter Institute, Rockville, MD, United States
[5]National Institutes of Health, NIAID, Laboratory of Biochemistry and Genetics, Bethesda, MD, United States
[6]University of Veterinary Sciences, Central European Institute of Technology (CEITEC), Brno, Czech Republic
[7]University of Veterinary Sciences, Faculty of Veterinary Medicine, Brno, Czech Republic
[8]ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, Laboratoire de Santé Animale, Maisons-Alfort, France
[9]IDEXX Laboratories, Kornwestheim, Germany
[10]German Federal Institute for Risk Assessment, Department for Biological Safety, Berlin, Germany
[11]Veterinary Research Institute, Hellenic Agricultural Organisation-Demeter, Thessaloniki, Greece
[12]Veterinary Research Institute, Department of Parasitology and Invasive Diseases, Pulawy, Poland
[13]National Institute of Public Health, Centre of Epidemiology and Immunology, Prague, Czech Republic
[14]University Bern, Institute of Parasitology, Bern, Switzerland
[15]Italian National Institute of Health, Rome, Italy
[16]Statens Serum Institut, Copenhagen, Denmark

Introduction:

Toxoplasma gondii is a highly prevalent protozoan parasite that globally infects a broad range of animals, including humans. A better knowledge of the genetic diversity and population structure of *T. gondii* may help to understand transmission routes and sources of infection for livestock and humans.

Objectives:

There is limited data on genome-wide comparisons of field isolates belonging to the same *T. gondii* clonal lineage. Therefore, the aim of the present study was to assess genome-wide genetic diversity among *T. gondii* Type II isolates from Europe, where this clonal lineage is predominant.

Materials and Methods:

The genomes of 75 European *T. gondii* Type II isolates, were assessed by whole genome sequencing (WGS) and compared to the Type II reference strain ME49.

Results:

At least 95% of the reads for each *T. gondii* European field isolate mapped to the reference whole genome sequence. Mapped reads covered over 99% of the reference genome with a read depth of > 70 per base. The total number of SNPs relative to reference varied between ~4000 and ~12000. Affinity propagation clustering (APC) analysis revealed 5 subpopulations among 75 *T. gondii* Type II isolates from Europe, whereas ADMIXTURE analysis showed the presence of 3 subpopulations. Subpopulations identified by both, APC and ADMIXTURE corresponded to each other. Interestingly, subpopulations were not equally distributed all over Europe but showed clustering in particular European regions.

Conclusion:

The European *T. gondii* Type II population is genetically more diverse than expected. Based on our experiences, WGS may be useful to better understand the molecular epidemiology of the parasite in Europe, to trace infection sources in outbreaks and to detect the introduction of exotic or the emergence of recombinant strains.

Funding

This work was done as part of TOXOSOURCES project, supported by funding from the

European Union"s Horizon 2020 Research and Innovation programme under grant agreement No

773830: One Health European Joint Programme.

A46

From endocytosis to recycling, the plasma membranes dynamics of the apicomplexan parasite *Toxoplasma gondii*

J. von Knoerzer-Suckow[1], *S. Gras*[1]

[1]Ludwig-Maximilians-Universität (LMU) , Lehrstuhl für Experimentelle Parasitologie, Planegg-Martinsried, Germany

The plasma membrane is at the interphase of the intracellular content and the environment. It plays a critical role in numerous functions from motility to cell communication. This is particularly true for the intracellular parasites, which are switching between drastically different environments during their life cycle. Endocytosis is an important key mechanism involved in the regulation of the plasma membrane surface protein and uptake of environmental molecule. In apicomplexan parasites, endocytosis has mainly been studied in the genus *Plasmodium spp*. Only recently, endocytosis has been successfully described in *Toxoplasma gondii* (*T.gondii*). The two types of endocytic mechanisms have been described in *T.gondii*: the uptake of proteins from the host cell and the uptake of plasma membrane proteins of the parasite. Previously, we showed the uptake of fluorescent lipids and antibodies in extracellular tachyzoites. We have now designed a new assay, which does not rely on exogenous material and allows us to image not only endocytosis but the whole plasma membrane dynamics. The gene coding for SAG1, one of the major surface antigens, was endogenously tagged with Halo. Using a new dual (spatial and/or temporal) labelling strategy, we were not only able to differentiate between internal and surface proteins, but also from mother and de-novo synthesised material during replication. We observed during replication that the plasma membrane from the mother is shared with the daughters, endocytosed, and recycled. We quantified this dynamics by tracking the changes in the fluorescence signals. Moreover, we identify for the first time that *T. gondii* generates extra plasma membrane "blebs" prior to the generation of the daughter cells as a reservoir for daughter cell hatching from the mother. In collaboration with the Waller"s laboratory, we have identified an important complex involved in endocytosis. Interestingly, inhibition of endocytosis also leads to a strong alteration of the plasma membrane dynamics. Taken together these data indicate that the reuptake of the plasma membrane is a critical step during replication.

A47

Late embryogenesis abundant proteins contribute to *Toxoplasma gondii* oocyst resilience to environmental stresses

D. Warschkau[1], D. Arranz-Solís[2], B. T. Fabian[1], J. P. Saeij[2], F. Seeber[1]

[1]Robert-Koch-Institute, Mycotic and Parasitic Agents and Mycobacteria (FG16), Berlin, Germany
[2]University of California Davis, Pathology, Microbiology and Immunology department, School of Veterinary Medicine, Davis, CA, United States

Introduction

Toxoplasma gondii oocysts are highly infectious, shed in large quantities by infected felines, are extremely stable in the environment and resistant to most inactivation procedures. Despite being a critical life stage for *T. gondii* transmission, the genetic basis for the environmental resistance of the oocysts and the contained sporozoites is largely unknown.

Objectives

Our objective was to test whether a set of sporozoite-specific proteins could determine *T. gondii* oocyst resilience to environmental stressors. Among them, we decided to investigate Late Embryogenesis Abundant (LEA)-related proteins, since LEAs are known to provide resistance to environmental stresses such as drought, salinity, and freezing in a variety of organisms. All four *T. gondii* LEA genes are located in a cluster (LEAc) on chromosome XII.

Materials & methods

We studied the cryoprotective effect of the four LEAs on *T. gondii* lactate dehydrogenase *in vitro* using recombinant proteins and assessing aggregation after repeated freeze-thaw cycles. Using an inducible expression system in *E. coli*, we tested whether the proteins confer resistance to low temperatures. Through biochemical and bioinformatic approaches, we analyzed the LEAs for features of intrinsically disordered proteins. By applying the CRISPR/Cas9 technology, we generated a LEAc knockout in a cat-compatible ME49 strain, and assessed the oocyst sensitivity to environmental stresses compared to that from wild-type parasites.

Results

Recombinantly expressed LEA proteins showed cryoprotective effects on lactate dehydrogenase *in vitro* and induced expression in *E. coli* improved viability at low temperatures. *T. gondii* LEAs showed features of intrinsically disordered proteins and amphoteric repeats, explaining some of the protein"s properties. Remarkably, LEAc knockout oocysts were more susceptible to high salinity, freezing and desiccation compared to oocysts from wild-type parasites.

Conclusion

We show for the first time that LEA proteins are important for the environmental resistance of *T. gondii* oocysts. Future studies using knockout strains for each of the LEA genes alone or in combination are now warranted to elucidate which LEAs are needed for the resistance phenotype.

A48

Dual use of *Toxoplasma gondii* mitochondrial DNA sequence block boundaries gives rise to short non-coding RNAs

S. Tetzlaff[1], A. Hillebrand[1], S. Wicke[2], C. Schmitz-Linneweber[1]

[1]Humboldt University of Berlin, Molecular Genetics, Berlin, Germany
[2]Humboldt University of Berlin, Biodiversity and Evolution, Berlin, Germany

1. Introduction

Mitochondrial genomes of apicomplexan parasites have only three open reading frames (ORFs) encoding subunits of the mitochondrial electron transport chain and in addition several genes for highly fragmented ribosomal RNAs. How Apicomplexan mitochondrial RNAs are expressed and processed, in particular how rRNA fragments are produced from precursor RNAs, is largely unknown. *Toxoplasma gondii* displays one of the most striking and poorly understood Apicomplexan mitogenome organization, built of 21 sequence blocks arranged in different combinations. How these blocks are combined has not been quantitatively analyzed so far.

2. Objectives

Generate a full-genome map of the mitochondrial genome of *T. gondii*.
Use the map to allow a comprehensive analysis of short RNAs in *T. gondii* – the first Apicomplexan transcriptome-wide sRNA analysis outside of *Plasmodium*.

3. Materials & methods

We established a protocol based on differential membrane permeabilization that allows purification of organellar DNA of *T. gondii*. Using single molecule Nanopore sequencing of mitochondrial DNA, we generated a comprehensive genome map of the variety of existing mitochondrial DNA molecules. Illumina-based short RNA (sRNA) sequencing was used to determine the first mitochondrial transcriptome of *T. gondii*.

4. Results

Our Nanopore sequencing efforts lead to a quantitative understanding of sequence block combinations in the *T. gondii* mitochondrial genome. We show that there are striking differences in the frequencies of block combinations and that 5"-ends of coding regions are linked to each other within the genome. sRNA sequencing data confirmed the transcription of previously predicted rRNAs and in addition identified a large number of novel noncoding short RNAs. Several of the newly discovered transcripts are spanning the borders of sequence blocks. This leads to the intriguing situation that - depending on the different DNA block combinations - chimeric sRNAs are generated at block borders. sRNAs with fusions of coding sequences with rRNA sequences are observed.

5. Conclusion

T. gondii is using block borders to produce short RNAs with overlapping but not identical sequences. This may lead to dual use of sequence elements as coding region and at the same time also for ribosome production.

A49

Geno- and phenotyping of the liver fluke (*Fasciola hepatica*) for comparative host-parasite genotype interaction studies

A. S. Hecker[1], M. K. Raulf[1], A. Strom[2], K. May[2], S. König[2], C. Strube[1]

[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Hannover, Germany
[2]Justus-Liebig-University Gießen, Institute of Animal Breeding and Genetics, Gießen, Germany

The liver fluke *Fasciola hepatica* is among the most important endoparasites in ruminants worldwide causing clinical symptoms and production losses. Anthelmintic treatment is widely implemented, but reports on resistance are increasing and the development of a vaccine has not been successful so far.

This project aims at investigating the cattle-*F. hepatica* interaction by simultaneous modelling of host- and parasite-derived genomic, proteomic and glycomic data to unravel novel approaches for prevention and control of *F. hepatica*.

To this end, Holstein-Frisian dairy cows from preselected herds are sampled at the abattoir and various infections traits were recorded. All liver flukes present in the bile ducts were measured, will be genotyped and variations in protein and glycan composition of the surface tegument are currently investigated via SDS-PAGE and immunoblot-based assays.

Out of the sampled animals (n=156), 53 cows showed patent *F. hepatica* infections with a mean fluke count of 5.5 flukes/liver. Investigation of the surface tegument revealed minor variations between individual flukes and major differences in glycosylation patterns compared to published data from Ireland, suggesting considerable variations between fluke populations.

Finally, all data will be considered simultaneously in genotype-by-genotype interaction analyses, which will provide new insights into host tolerance/resistance induction to unravel potential candidates for future vaccine development.

A50

Winners vs. Losers – Comparative transcriptomic analysis of *Schistosoma mansoni* mature and immature eggs from gut and liver

L. Konečný[1,2], K. Peterková[1,2], M. Sombetzki[3], J. Dvořák[2,4]

[1]Charles University Prague, Department of Parasitology, Prague, Czech Republic
[2]Czech University of Life Sciences, Center of Infectious Animal Diseases, Prague, Czech Republic
[3]Universitätsmedizin Rostock, Zentrum für Innere Medizin, Abteilung für Tropenmedizin, Infektionskrankheiten und Sektion Nephrologie, Rostock, Germany
[4]Czech Academy of Sciences, Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic

Introduction

The eggs of *Schistosoma mansoni* are the main cause of the clinical manifestations of schistosomiasis. It is important to note, however, that only the egg "losers" trapped in the host tissues, are responsible for these pathologies. After laying, the egg "winners", on the other hand, manage to attach to the endothelium of the mesenteric vein, and after a period of development, induce the growth of a granuloma which facilitates their passage through the intestinal wall to gut lumen. "Losers" carried with a blood stream to non-specific tissues also undergo full development and induce a granuloma formation, but their life ends there. Although trapped eggs represent a dead end in the life cycle, vast majority of transcriptomic and proteomic studies attempting to describe the biology of the eggs have studied these liver-trapped egg "losers" instead of gut-attached egg "winners".

Objectives

To assess if and how the gene expression of the egg is affected by the surrounding tissues.

Materials & Methods

We isolated eggs from the liver and intestinal tissues of experimentally infected mice, divided eggs into mature and immature and compared their transcriptomic profiles.

Results

Our results clearly show that gene expression in *S. mansoni* eggs is critically dependent on tissue localization. In addition to the crucial differences between eggs derived from the two tissues, the expression profiles of liver-derived eggs are very similar regardless of their developmental stage, whereas gut-derived eggs show remarkable changes during their maturation. The most differentially expressed genes of interest are the Venom allergen-like proteins (VALs), which, together with proteases and protease inhibitors, are substantially more active in intestinal eggs. In stark contrast, IPSE/alpha-1, omega-1, as well as the majority of micro-exon genes (MEGs), which are often discussed as the primary weapons of the egg-winners, are, in fact, restricted to liver losers.

Conclusion

We argue that such differential expression of many important groups of molecules directly reflects the environment in which the egg is located. While in the case of the gut-derived eggs, who attach themselves to the cell wall, the up-regulated molecules probably represent the tools for successful passage to the external environment, in the case of the liver-derived eggs, the specific expression is more likely a response to host defence systems and thus reflects the failure of these individuals.

Fig. 1

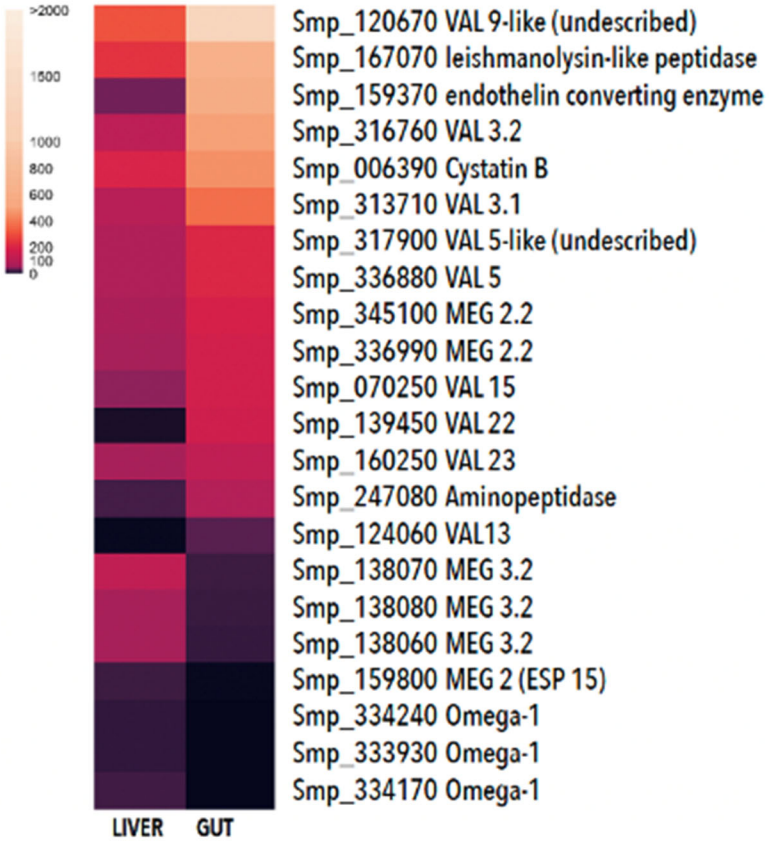


Figure 4: Selection of significantly (padj<0.05) differentially (log2FC >1) expressed genes in mature gut vs. liver eggs. Analysis done with Deseq2 R package. Values represented in reads per million (RPM).

Fig. 2

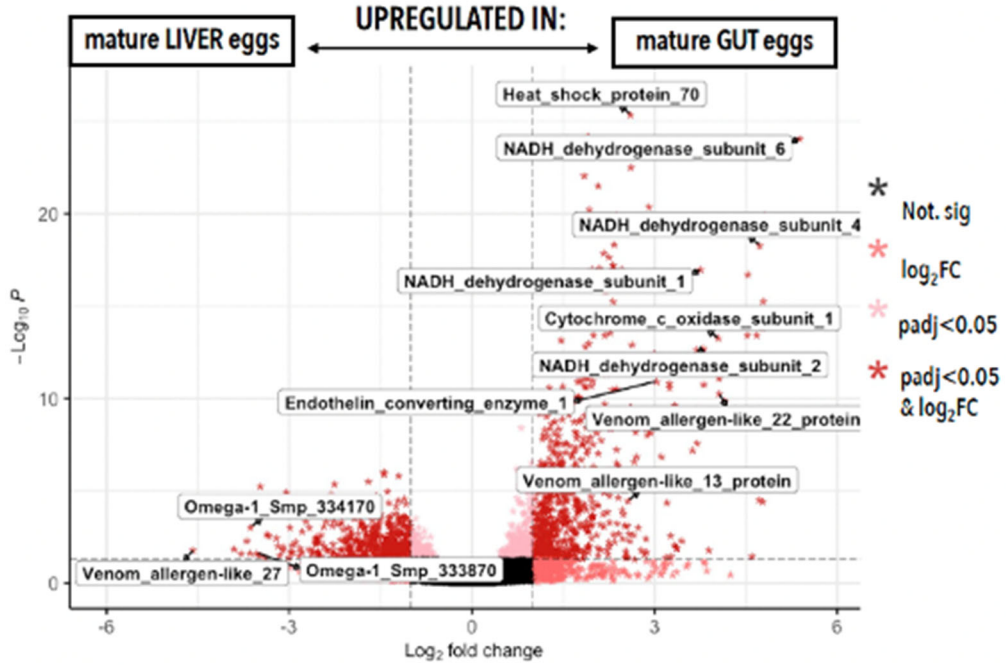


Figure 2: Volcano plot – upregulated genes in mature LIVER (left) vs mature GUT (right) eggs.

A51

Long-term unisexual infection with *Schistosoma mansoni* in mice has the potential to boost the immune response to oviposition of challenge infection

C. Reinholdt[1], F. Winkelmann[1], N. Koslowski[1], E. C. Reisinger[1], M. Sombetzki[1]

[1]University Medical Center Rostock, Department of Tropical Medicine and Infectious Disease, Rostock, Germany

Background: The complexity of *Schistosoma* spp. life cycle and their highly effective immune evasion strategies, makes vaccine development challenging. Unisexual infection, excluding immunomodulatory parasite eggs, offers a distinct perspective on host immune responses to both worm sexes and thus may provide a better understanding of complex immunological processes and identification of new targets for vaccine research. We have recently shown that in mice, a long-term unisexual infection with schistosomes leads to an unpolarized Th1/Th2 response associated with an abnormally enlarged spleen and diffuse hepatic inflammation. Aim: In this study, we analyzed whether unisexual worms can mate after 3 months of single sex infection and thus the Th2 response induced by oviposition can reverse or heal the described systemic inflammation. Methods: Mice were infected with male or female cercariae and reinfected with the opposite sex after 12 weeks for the same period. Unisexually infected mice and bisexually infected mice served as controls. At 24 weeks after initial infection, we histologically examined worm mating as evidenced by presence of eggs, infection-related pathology associated with eggs and characterization of liver fibrosis. We analyzed the immune status of the livers and spleens by using flow cytometry, recorded blood cell composition, and performed gene expression analysis of the livers. Results: We found that unisexual worms find each other, mate, and start oviposition even after 3 months. However, we observed more severe hepatic fibrosis in the reinfected groups compared to the bisexually infected group. Egg deposition has been associated with a typical Th2 immune response in the liver after reinfection, along with increased CD4+ T cell immune cells recruitment. Our results show that eggs are able to restore the Th1/Th2 immune imbalance of a previous unisexual infection. However, the organ damage caused by the unisexual worms does not subside, but rather provides the basis of egg-induced inflammation. Conclusion: Schistosomes can survive unpaired for a long time and cause severe organ damage. Given that schistosomes can mate several months after unisexual infection and then worm- and egg-related organ damage can accumulate, infection status without egg proof must be considered. Another critical issue is the tendency to crosshybridization of human schistosomes with livestock specific species, which can further contribute to the spread of the parasite.

A52

Utilisation of "Omics" to unmask the interactions of adult male and female *Schistosoma mansoni* with their host

F. Winkelmann[1], M. Gesell Salazar[2], C. Hentschker[2], S. Michalik[2], C. Schulz[1], M. Bischofsberger[1], E. C. Reisinger[1], U. Völker[2], M. Sombetzki[1]

[1]Rostock University Medical Center, Department of Tropical Medicine and Infectious Diseases, Rostock, Germany
[2]University Medicine Greifswald, Interfaculty Institute for Genetics and Functional Genomics, Department of Functional Genomics, Greifswald, Germany

Introduction: *Schistosoma* spp. are gonochoristic trematodes that cause one of the most devastating worm parasitoses in the world. To date, there is no effective vaccine to protect against the infection, and drug treatment with praziquantel has limited success in endemic areas. In the search for new therapeutic approaches to combat this disease, adult female schistosomes have so far been neglected because they have little direct contact with the environment and thus with the host during their mating with male worms.

Objectives: Using "Omic" technologies, we aim to study the interactions between adult male and female Schistosoma mansoni and their host to find targets for new therapeutic strategies.

Materials & methods: First, we studied the structure of the tegument of male and female unpaired and paired schistosomes after contact with human serum as the host interface. Comparative analyses were performed by electron microscopy and immunohistochemistry. In addition, the tegument proteome of male and female unpaired and paired schistosomes was examined using a novel and highly sensitive workflow by LC-MS/MS analysis. In addition, the effect of circulating antigens from male and female schistosomes on the host immune response was investigated. For this purpose, the transcriptome and immune cell populations in the spleens of unisexually infected mice were analyzed.

Results: After incubation in human serum, adult male and female schistosomes exhibited marked surface enlargement, and females showed shedding of their outer surface. Using proteomic analyses, we identified 1519 tegument proteins for male and female unpaired and paired schistosomes. We identified more proteins in male than in female worms, regardless of whether they were derived from mating or from unisexual infection. For transcriptomic analyses, 22,207 transcripts were examined in the spleens of unisexually infected mice. Principal component analysis showed clear clustering of experimental groups. Our studies suggest that male and female S. mansoni elicit an egg-independent, non-polarized Th1/Th2 immune response in the host, with males having a significantly greater immunoregulatory influence on gene regulation in the spleen and thus on the host.

Conclusion: Our initial prelimiary findings need to be investigated to discover and understand new metabolic pathways and immunomodulatory mechanisms of adult worms to contribute to the development of new therapeutic strategies against schistosomiasis.

A53

Egg secreted proteins: greater diversity than we thought

J. Dvořák[1,2], L. Konečný[3], M. Sombetzki[4], K. Peterková[3]

[1]University of Life Sciences, Center of Infectious Animal Diseases, Prague, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic
[3]Charles University Prague, Parasitology, Prague, Czech Republic
[4]Universitätsmedizin Rostock, Zentrum für Innere Medizin, Abteilung für Tropenmedizin, Infektionskrankheiten und Sektion Nephrologie, Rostock, Germany

Introduction: The eggs produced by *Schistosoma mansoni* adults are responsible for the pathology in the host tissues. Immature eggs bind to the endothelial surface and until that point, they are immunologically inert. When mature, attached eggs actively induce host inflammatory processes to pass from the vasculature endothelial tissue into the gut environment. They evoke granuloma tissue formation - a highly organized multicellular cluster enriched with immune cells. Many eggs are washed out from mesenteric veins mostly to the liver, where granuloma formations cause significant pathologies.

Objectives: So far, little is known about the capability of the excretory/secretory products of eggs to stimulate these processes. Only a few secreted molecules have been experimentally studied and had their function revealed. So far, most of the attention has been devoted to egg-specific glycoproteins IPSE-1/alpha-1 and omega-1, among others promoting Th2 polarization. Nevertheless, the entire molecular composition of egg secretome and the roles of particular molecules remain unresolved.

Materials & methods: We are currently analyzing a portfolio of molecules produced by *S. mansoni* eggs by combining transcriptomic analysis and biochemical approaches. We focused on developed and undeveloped eggs isolated from the gut and liver tissue. Moreover, eggs of different *S. mansoni* geographical isolates are being analyzed in detail by bioinformatic pipeline in our lab as we have evidence that significant differences in the inflammatory reactions of rodent models are present.

Results & conclusion: Our preliminary data pointed out that gene expressions differ significantly based on the developmental stage of the eggs, tissue localization and *S. mansoni* strain. Our findings especially highlight two groups of proteins, the micro-exon gene (MEG) family and venom allergen-like (VAL) proteins, which are the most abundant molecules in egg transcriptome and their presence is stage and tissue localization dependent. Multi-isoform schistosome-specific proteins from MEG family encode some of the major secreted proteins with unknown functions with ~80% of the protein-coding region comprising short symmetric exons from 6-36 bp. VAL family shares a unique SCP/TAPS protein domain, which varies in length between 120 and 170 amino acids. Detailed immunohistochemical and biochemical studies are ongoing.

A54

Identifying mechanisms of preventing immunopathology during *Trypanosoma brucei* infection

M. Shi[1], *G. Liu*[1]

[1]University of Maryland, Veterinary Medicine, College Park, MD, United States

Introduction:

Induction of a robust immune response is essential for hosts to fight microbial infections. However, excessive immune responses without the presence of appropriate regulations also damage tissues, leading to immunopathology and death during infections. We have previously shown that IL-27 signaling is required for survival during African trypanosome infection by preventing lethal effect of $CD4^+$ T cells and IFN-γ. However, the cellular and molecular mechanisms involved in preventing immunopathology still remain incompletely understood.

Objectives

to determine the cells and molecules involved in regulating immunopathology during *T. brucei* infection.

Materials & methods:

Wild-type and knockout mice were infected with *T. brucei*. Flow cytometry, ELISA, and adoptive transfer experiments were performed to analyze the immune response in the infected mice.

Results

We found that $CXCR6^{-/-}$ mice infected with *T. brucei* survived significantly longer than infected wild-type mice, associated with demilished liver immunopathology and reduced pro-inflammatory cytokines including IFN-γ and TNF-α. Adoptive transfer of wild-type $CD4^+$ T cells, but not $CXCR6^{-/-}$ $CD4^+$ T cells, significantly reduced the survival of infected $CXCR6^{-/-}$ mice, demonstrating that $CXCR6^+CD4^+$ T cells mediated the early mortality of infected mice. Interestingly, mice deficient in LFA-1, an integrin molecule that is continuously expressed on $Foxp3^+$ Tregs, died significantly earlier than wild-type mice during *T. brucei* infection, associated with enhanced activation of $CD4^+$ T cells and higher secretion of IFN-γ. Importantly, infected $LFA\text{-}1^{-/-}$ mice exhibited significantly lower frequency and absolute number of $Foxp3^+$ Tregs.

Conclusion

$CXCR6^+CD4^+$ T cells promote early mortality of mice infected with *T. brucei*, while LFA-1 is essential for preventing the early mortality of infected mice, likely by maintaining $Foxp3^+$ Tregs numerically and functionally.

A55

Parasitic molecular triggers of coccidia-induced NETosis: a role for cell surface glycans

I. Conejeros[1,2], Z. Velásquez[1,2], G. Espinosa[1,2], C. Hermosilla[1,2], A. Taubert[1,2]

[1]Justus-Liebig-Universität Gießen, Gießen, Germany
[2]Institute of Parasitology, Gießen, Germany

Introduction

Coccidian parasites represent an important threat for animal and human health. Infection-driven innate immune reactions of the hosts depend on the adequate recognition of parasites. One major mechanism relies on the recognition of parasite-own pathogen associated molecular patterns (PAMPs). Among PAMPS, glycan residues on the parasite"s surface are one of the most important drivers of host immune responses. Polymorphonuclear neutrophils (PMN) are one of the first immune cells to arrive at the site of injury or infection. PMN-derived effector mechanisms against coccidian parasites include reactive oxygen species (ROS) production, degranulation and neutrophil extracellular trap (NET) formation. In this study, the glycosylation patterns in *Besnoitia besnoiti* and *Toxoplasma gondii* tachyzoites in addition to *Eimeria bovis* sporozoites and the relevance of different glycans in *B. besnoiti*- and *T. gondii*-induced NETosis were studied.

Material and Methods

Glycan pattern in *B. besnoiti* and *T. gondii* tachyzoites as well as *E. bovis*-sporozoites were determined by lectin blots using the following lectins: GNA, SNA, MAA, DSA, PNA. Blockage and modification of *B. besnoiti* and *T. gondii* tachyzoite-related glycan motifs was achieved by chemical treatments (NaIO4 oxidation), enzymatic treatments with PNGase F, PNGase A and O-glycosidase or by parasite culture in human foreskin fibroblasts (HFF) treated with the N-glycosylation inhibitor tunicamycin. After treatments, *B. besnoiti* and *T. gondii* tachyzoites were confronted with bovine PMN and NET formation was analyzed by confocal microscopy. Percentages of NET formation were calculated by image analysis.

Results and conclusions

Depending on the lectin used, overlapping and differing glycan pattern were revealed in tachyzoite (*B. besnoiti*, *T. gondii)* and sporozoite (*E. bovis)* stages by lectin blots. In contrast to tachyzoites, GNA-detected N-glycans proved absent in *E. bovis* sporozoites. Glycan oxidation via NaIO4 and PNGase F treatments led to a decrease in NET formation, but also affected tachyzoite viability. In the case of tunicamycin-treated HFF, *T. gondii* but not *B. besnoiti* tachyzoites were able to develop F2 tachyzoite generation. F2-tachyzoites proved impaired in their glycosylation repertoire and induced less NETs than those originating from untreated control HFF layers, thereby indicating a potential role of N-glycans in coccidia-induced NETosis.

A56

P2X1 receptor antagonist NF449 inhibits *Besnoitia besnoiti*-induced neutrophil clustering and anchored extracellular trap (NET) formation

G. Espinosa[1], *I. Conejeros*[1], *C. Hermosilla*[1], *A. Taubert*[1]

[1]Parasitology, Gießen, Germany

Introduction

Bovine besnoitiosis is a re-emerging cattle disease caused by the apicomplexan parasite *Besnoitia besnoiti*. Neutrophil extracellular traps (NETs) formation represents an efficient innate immune mechanism of polymorphonuclear neutrophils (PMN) against parasites, including *B. besnoiti*. PMN-derived purinergic signaling was proposed as critical factor for NET formation. ATP is an important purinergic ligand, which regulates several PMN actions, such as chemotaxis, ROS production and NET formation, via the P2 nucleotide receptor family. However, little data are available on the role of ATP as key modulator of purinergic signaling in *B. besnoiti*-triggered NETosis.

Material and methods

Bovine PMN were isolated from the blood of healthy cows by density gradient and exposed to *B. besnoiti* tachyzoites in the presence of ATP and purinergic receptor antagonists. Total and extracellular ATP levels were quantified by luminometry. NET formation was estimated as extracellular DNA by picogreen-derived fluorescence intensities. PMN oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) were quantified by Seahorse instrumentation. NET and cluster formation of PMN exposed to *B. besnoiti* was evaluated by epifluorescence microscopy.

Results

Current data showed that *B. besnoiti* tachyzoite exposure neither changed total ATP levels in PMN nor in the extracellular environment even though significantly triggering NET formation. Interestingly, exogenous supplementation of ATP failed to affect *B. besnoiti*-induced OCR but led to an increase of ECAR in PMN. Likewise, parasite exposure enhanced ECAR in PMN. However, neither the latter effect nor tachyzoite-triggered total NET formation was influenced by exogenous ATP levels. *B. besnoiti*-induced anchored NET formation was selectively blocked by the inhibition of the purinergic receptor P2X1 whilst antagonists of P2Y2, P2Y6, P2X4 and P2X7 receptors all failed to affect parasite-driven NETosis. [AT1] As an interesting finding, we additionally observed that tachyzoites exposure increased the percentage of PMN forming clusters and that this effect was P2X1-dependent.

Conclusion

B. besnoiti induces an increase in clustering, oxidative activity and NET formation in bovine PMN. We identified the P2X1 as a pivotal purinergic receptor to be involved in *B. besnoiti*-induced anchored NET formation therefore suggesting this receptor as an interesting target for future treatments of NET-mediated tissue damage.

A57

Interactions of human dendritic cells with *Cryptosporidium parvum*

R. Ross[1], S. S. Hasheminasab[2], I. Conejeros[2], U. Gärtner[3], F. Kamena[4], A. Krueger[1], A. Taubert[2], C. Hermosilla[2]

[1]Institute of Immunology, Gießen, Germany
[2]Institute of Parasitology, Gießen, Germany
[3]Institute of Anatomy and Cell Biology, Gießen, Germany
[4]Department of Microbiology and Parasitology, Buea, Cameroon

ntroduction: Little is known on *Cryptosporidium parvum*-driven responses of innate immune cells, such as PMN, monocytes, or macrophages, and even less is reported on parasite interactions with dendritic cells (DCs). DCs are sentinel cells residing in nearly all tissues including the small intestine. DCs detect pathogenic microorganisms with a broad range of pattern recognition receptors, take up, process and present antigens and migrate to the lymphoid organs to present them to naïve T cells. They decide, moreover, based on the information obtained, which kind of immune response like Th1-, Th2-, Treg-, or Th17- is elicited.

Objectives: We here intended to analyse early responses of human DCs driven by *C. parvum* stages.

Materials and methods: Immature human myeloid DCs were generated from monocytic blood precursors derived from buffy coats using GM-CSF and IL-4. Cells were harvested on day 6 of culture and exposed to medium alone (negative control), *C. parvum* oocysts/sporozoites and/or LPS (positive control) over night. Phagocytosis assays were conducted as well as scanning electron microscopy (SEM) analysis to unveil interactions between DCs and these two *C. parvum* parasite stages. Flow-cytometry analysis (FACS) demonstrated not only upregulation of classical DC activation markers and costimulatory molecules required for T cell-stimulation after *C. parvum* exposure but also phagocytosis. Cytokine IL-6 and chemokine IL-8 production in *C. parvum*-exposed human DCs was analysed by ELISAs. Finally, live cell 3D holotomography microscopy (Nanolive) was performed for visualization of highly motile DCs during first encounters with *C. parvum*.

Results: SEM shows not only DCs entrapping oocysts/sporozoites with their dendrites but also phagocytizing them. *C. parvum* induced IL-6- and IL-8- secretion in stimulated human DCs. Human DCs seem capable to phagocyte efficiently both stages of *C. parvum* (i. e. oocysts and sporozoites). Positive control (LPS stimulation) and *C. parvum* both upregulated DC maturation markers, i. e. CD83, antigen-presentation molecules (HLA-DR, CD1a), costimulatory molecules (CD40, CD86) and adhesion molecules (CD11b, CD58) as required for eliciting a strong adaptive immune response.

Conclusions: Considering that a strong adaptive immune response is required to protect against *C. parvum* infection, we investigated how DCs interact with *C. parvum* to open a new avenue in the search for an effective medication against this zoonotic parasite.

A58

Mechanisms of aggravation of cutaneous leishmaniasis by iron overload

S. Obermeyer[1], U. Schleicher[1], C. Bogdan[1]

[1]Institute of Clinical Microbiology, Immunology and Hygiene, Universitätsklinikum Erlangen and FAU Erlangen-Nürnberg, Erlangen, Germany

Iron overload can cause increased susceptibility to certain intracellular infectious pathogens, but the exact underlying mechanisms remain poorly defined. Here, we investigated how iron loading affects the disease outcome in self-healing C57BL/6 mice infected with the intracellular protozoan parasite *Leishmania (L.) major*.

Iron-loaded *L. major*-infected C57BL/6 mice showed disease exacerbation with aggravated skin swelling at the site of infection and higher parasite loads in the lesion. This was paralleled by enhanced cell death in the infected tissue, increased influx of CD11b+Ly6G+Ly6C+ neutrophil-like cells lacking suppressor activity and decreased percentages of T-cells, eosinophils, macrophages and dendritic cells. Furthermore, iron overload increased the expression of IFN-gamma, TNF, IL-1ß, IL-4, IL-17A, TGF-beta and type 2 nitric oxide synthase (NOS2) mRNA in skin lesions, whereas NOS2 protein expression and NO production in vivo was suppressed compared to control mice. In vitro, iron partially rescued *L. major* from NO-dependent, but ferroportin-independent killing by macrophages and favored the differentiation of Th2 cells, which was due to an oxidant-dependent increased nuclear translocation of STAT6. Arginase (Arg) 1, which competes with NOS2 for the substrate L-arginine, was upregulated on mRNA and protein level. In C57BL/6 mice lacking Arg1 in hematopoietic and endothelial cells, the aggravated course of disease following iron loading was abolished and NO production in the skin lesions was partially restored. Similar effects were observed, when iron-loaded C57BL/6 mice were treated with anti-IL-4-antibodies, indicating that iron-driven Arg1 induction is mainly due to enhanced IL-4 levels.

Thus, iron overload differentially affects NOS2 and Arg1 expression. Disease exacerbation and impaired parasite control by iron excess in the infected tissues at least partially result from an oxidant-dependent expansion of Th2 cells and an IL-4-mediated Arg1 expression that decreases NO production in iron-loaded mice.

A59

Arginase 1 causes chronicity of *Leishmania mexicana* infection via the generation of a replicative monocytic host cell niche

B. Rai[1], L. Chunguang[2], A. Debus[1], M. Jeninga[1], C. Daniels[3], S. Roberts[4], M. Rauh[5], J. Mattner[1], M. Kunz[6], C. Bogdan[1], U. Schleicher[1]

[1]Institute of Clinical Microbiology, Immunology and Hygiene, Universitätsklinikum Erlangen and FAU Erlangen-Nürnberg, Erlangen, Germany
[2]Department of Bioinformatics, Biozentrum, Universität Würzburg, Würzburg, Germany
[3]Department of Nephropathology, Universitätsklinikum Erlangen, Erlangen, Germany
[4]Pacific University, Department of Pharmaceutical Sciences, Forest Grove, OR, United States
[5]Clinical Laboratory, Department of Pediatrics, Universitätsklinikum Erlangen, Erlangen, Germany
[6]Chair of Medical Informatics, FAU Erlangen-Nürnberg, Erlangen, Germany

Background and objective

Control of *Leishmania* (*L*.) parasites require interferon (IFN)γ-dependent type 2 nitric oxide synthase (NOS2), an enzyme that converts L-arginine into citrulline and nitric oxide (NO). NOS2 activity is counteracted by arginase (ARG) 1 and 2, both of which are induced by Th2 cytokines and cleave L-arginine into urea and ornithine, a precursor of polyamines. Recently, we observed that the expression of ARG1 and ARG2 steadily increased in *L. mexicana*-infected BALB/c and C57BL/6 mice during disease manifestation. Here, we studied the role of host cell arginases during *L. mexicana*-induced chronic cutaneous leishmaniasis (CL).

Methods

C57BL/6 wild-type (WT), germ-line k.o. (Arg2-/-) or cell-specific conditional k.o. mice (Arg1ΔTek, Arg1ΔCx3cr1, Il10ΔCd4) were infected into the skin with 3×106 *L. mexicana* promastigotes. Infected skin tissue was processed for mRNA/protein expression, metabolomics and single-cell (sc) RNAseq analyses at different time-points post infection (p.i.).

Results

C57BL/6 WT mice developed non-healing chronic CL, whereas Arg2-/- mice showed progressive disease with a delayed onset. Deletion of Arg1 in hematopoietic and endothelial cells (Arg1ΔTek) or in monocytes and macrophages (Arg1ΔCx3cr1) exhibited a strongly reduced pathology and ultimately resolved their skin lesions despite parasite persistence, suggesting that myeloid Arg1 accounts for chronic CL. Mice lacking IL-10 in CD4+ T cells (Il10ΔCd4) defined IL-10 as a factor inducing ARG1 during infection. Metabolomics revealed the depletion of L-arginine by ARG1 and a significant rise in polyamines in the infected WT skin and draining lymph nodes. At day 40 p.i., when Arg1 mRNA was already upregulated in WT mice, but disease manifestation, parasite burden and the metabolic profile were still comparable between WT and Arg1-deficient mice, similar levels of NO were seen in WT and Arg1-deficient mice, although the expression of NOS2 protein was much higher in WT mice. ScRNAseq analysis of skin lesion cells identified distinct myeloid subpopulations that were enriched in WT mice, including a prominent Arg1+/NOS2+ and IFNγ-dependent cluster of inflammatory macrophages.

Conclusion

ARG1 serves as an unexpected signal for the recruitment and differentiation of monocytes in the skin of *L. mexicana*-infected mice, which leads to an enhanced immunopathology and the generation of a cellular niche that favors parasite replication due to ARG1/NOS2 substrate competition.

Workshop II – Exit

A60

A proteomic view on the egress-related vesicles of malaria gametocytes

J. Sassmannshausen[1], S. Bennink[1], U. Distler[2], J. Küchenhoff[1], P. C. Burda[3], S. Tenzer[2], T. W. Gilberger[3], G. Pradel[1]

[1]RWTH Aachen University, Division of Cellular and Applied Infection Biology, Institute of Zoology, Aachen, Germany
[2]University Medical Centre of the Johannes-Gutenberg University, Proteomics Core Facility, Institute of Immunology, Mainz, Germany
[3]Centre for Structural Systems Biology (CSSB), Bernhard Nocht Institute, Hamburg, Germany

Introduction

Gametocyte egress from the host erythrocyte follows an inside-out mode during which the membrane of the parasitophorous vacuole ruptures prior to the erythrocyte membrane. Membrane rupture requires the exocytosis of specialized secretory vesicles of the parasites; i.e. the osmiophilic bodies (OBs) involved in rupture of the parasitophorous vacuole membrane, and vesicles, termed g-exonemes, that harbor the perforin-like protein PPLP2 needed for erythrocyte lysis. While several OB proteins are known, like G377 and MDV1/Peg3, the protein composition of the g-exonemes remains unclear.

Material/Method

We employed BioID analyses to unveil the interactomes of OBs and g-exonemes. BioID is a proximity labelling application for detecting putative protein-protein interactions. The method uses a promiscuous biotin ligase (BirA), fused to a bait protein that can be induced to biotinylate interacting and proximal proteins during a defined labelling period by biotin supplementation. TurboID represents an optimized version of the BioID, which uses a biotin ligase with higher activity and therefore decreases the labelling period dramatically. Biotinylated proteins can be isolated, analyzed via mass spectrometry (MS) and investigated as candidate interactors with the bait protein or as constituents within a subcellular domain.

Results

Here, we used high-resolution imaging and BioID methods to study the two types of egress vesicles in *Plasmodium falciparum* gametocytes. We show that OB exocytosis precedes discharge of the g-exonemes and that the two types of vesicles exhibit distinct proteomes. In addition to known egress-related proteins, our analyses revealed novel markers of OBs and g-exonemes, including components involved in protein processing and vesicle trafficking.

Discussion

Our data provide insight into the immense molecular machinery required for the inside-out egress of *P. falciparum* gametocytes.

A61

Intracellular *Leishmania major* parasites drive host cell death and cell-to-cell transfer depending on the parasite proliferation rate

I. Baars[1], A. J. Müller[1,2], G. van Zandbergen[3,4,5], M. Jaedtka[3]

[1]Otto-von-Guericke-University, Institute for Molecular and Clinical Immunology, Magdeburg, Germany
[2]Helmholtz Centre for Infection Research GmbH, Research Group Intravital Microscopy of Infection and Immunity, Braunschweig, Germany
[3]Paul Ehrlich Institute, Division of Immunology, Langen, Germany
[4]Johannes-Gutenberg University Mainz, Institute for Immunology, Mainz, Germany
[5]Johannes-Gutenberg University Mainz, Research Center for Immunotherapy, Mainz, Germany

Introduction: The virulence of the intracellular pathogens relies largely on the ability to survive and replicate within phagocytes, but also to be released and transferred into new host cells. Recent findings suggest that release of *Leishmania* parasites from infected host cells is strictly regulated, with the mechanisms involved still to be determined.

Objectives: To study *Leishmania major* (*L. major*) transmission from infected monocytes to newly recruited cells in the context of infected host cell death.

Materials & methods: We quantified *L. major* transfer and uptake of host cell material together with the parasite into adoptively transferred cells *in vivo* using intravital 2-photon microscopy analysis and flow cytometry analysis of CD11c-YFP reporter mice. Also, we visualized cell death by live cell imaging, and by intravital 2-photon imaging of a fluorescence resonance energy transfer-based (FRET-based) cell-death-biosensor.

Results: We observed increased original host cell material uptake in infected as compared to uninfected cells. This suggests that original host cells can be phagocytosed by new cells together with the parasites. Also, infected host cells show signs of cell death before parasite transfer *in vitro* and show more Caspase-3 activity, indicating apoptosis, compared to uninfected cells *in vivo*. Lastly, we detected higher *L. major* proliferation in TUNEL+ compared to TUNEL- murine intraperitoneal macrophages using a photoconversion-based proliferation biosensor *in vitro*. In addition, our data indicate that high parasite proliferation is associated to shorter infection time of the infected host *in vitro*.

Conclusion: These data suggest that a high *L. major* proliferation rate induces cell death and thereby drives the dissemination of the pathogen to new phagocytes.

A62

3D electron tomography of *Plasmodium*

Y. Sokolowski[1], B. He[1], L. Dorner[1], S. Heineken[1], A. Böhmert[1], M. Cyrklaff[1], F. Frischknecht[1]

[1]Heidelberg University, Center for Infectious Diseases, Heidelberg, Germany

The life cycle of *Plasmodium* includes a short phase of sexual reproduction in the mosquito. Immediately after uptake of the blood meal gametocytes are activated and start to differentiate. Male gametocytes produce eight male flagellated microgametes that egress from the erythrocyte with the help of axonemal movement. Axonemes are microtubule-based cytoskeletal structures essential for ciliary beating and flagellar motility. Canonical axonemes consist of nine doublet microtubules which are linked with dynein motors and nexin linkers and surround two central microtubules. Axonemal microtubules can polymerize from basal bodies, these are extracellular parts of the bipartite microtubule organizing center (MTOC) that connect the axoneme through a nuclear pore with the spindle. Here, we investigate gamete formation in wild-type and mutant parasites. The mutants lack the merozoite thrombospondin-related anonymous protein (MTRAP) and do not egress from the infected cell. Cells were fixated at different time points and serial sections were imaged by transmission electron microscopy to generate several 3D volumes. 3D datasets were segmented by surface rendering to generate 3D models. The generated 3D models reveal that *Plasmodium* axonemes are formed by a zipping process, cause nuclear fractioning and can feature several aberrations. We imaged both the connection and apparent rupture of the bipartite MTOC into basal bodies and the spindle pole, suggesting a model of axoneme formation. Overall, our data reveal that an astonishing level of inaccuracy stills allows rapid axoneme assembly and efficient gamete motility and fertilization.

A64

L. major hijacks hypoinflammatory monocytes to perpetuate infection in an interleukin-7 dependent feedback loop

Y. Fu[1], E. Vafadarnejad[2], I. Baars[1], A. E. Saliba[2], T. Schüler[1], A. J. Müller[1]

[1]Faculty of Medicine, Otto-von-Guericke-University Magdeburg, Institute of Molecular and Clinical Immunology, Magdeburg, Germany
[2]Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany

Introduction: *Leishmania major* (*L. major*) infection of the skin is marked by large heterogeneity in both pathogen physiological states and immune cell populations recruited to the infection site. Our previous work suggested that pathogen proliferation and cell-to-cell transfer is preferentially occurring in distinct phagocyte subpopulations. However, the link between pathogen physiology with the activation of infected host cells remains poorly understood.

Objectives: To investigate the dynamics of the recruitment, activation and deployment of cellular defence mechanisms of phagocytes recruited to the site of *L. major* infection in the context of differential pathogen proliferation rates.

Materials & Methods: Using an in vivo biosensor for *L. major* proliferation in combination with single cell RNAseq, we investigated transcriptional profiles of infected phagocytes isolated from infected skin dependently of the intracellular pathogen proliferation rate. Bone marrow chimeric mice, cell-specific depletion and cytokine blockade were used to characterize candidate cells and molecules with a possible impact on pathogen control using flow cytometry and multiparameter microscopy.

Results: We observed that fast versus slow *L. major* proliferation is linked to clearly defined infected cell populations with differential gene expression profiles. In particular, we identifed a distinct monocyte subpopulation, termed Lo3, which harboured low proliferating *L. major* and exhibited a hypoinflammatory phenotype. Furthermore, our data suggest that Interleukin-7 (IL-7), which is produced in the tissue upon induction of the immune response, acts as a feedback mechanism which restricts the immune response via the Lo3 monocytes. In particular, depletion of Lo3 monocytes, transient inhibition of IL-7, or absence of the IL-7 receptor on phagocytes drastically enhanced *L. major* clearance via an enforced T cell response.

Conclusion: *L. major* hijacks an immunoregulatory feedback mechanism which is mediated via IL-7 and hypoinflammatory monocytes which are specifically characterized by the infection of low proliferating pathogen. These cells seem to dampen the adaptive immune effector response and and perpetuate pathogen persistence in the tissue.

A65

A transdifferentiated human macrophage-like cell line to elucidate the cell-to-cell spread of *Leishmania major*

K. Volkmar[1], M. Jaedtka[1], B. Walber[1], K. Bagola[1], H. Heine[2], G. van Zandbergen[1,3]

[1]Paul-Ehrlich-Institut, Immunology, Langen, Germany
[2]Research Center, Leibnitz Lung Center Borstel Borstel,, Innate Immunity, Borstel, Germany
[3]Johannes-Gutenberg University Mainz, Immunology, Mainz, Germany

Leishmania is the causative agent of the tropical neglected disease leishmaniasis and it infects macrophages as its definitive host cell. While the routes of initial infection are well understood, the mechanism of parasite spread to other cells remains vague, partially due to lack of genome editing methods in human macrophages. Hence, we set up an infection model in BLaER1 cells, which can be edited on a genomic level and transdifferentiated into a macrophage-like phenotype. To proof their suitability as infection model, we first examined the immunophenotype of BLaER1 cells and found it to be comparable with macrophages. We also confirmed their susceptibility for the infection with *Leishmania* promastigotes using confocal microscopy. The infection rate and cytokine responses were very similar when comparing infected BLaER1 and macrophages. Focusing on host cell death as a possible mechanism of the cell-to-cell spread of *Leishmania*, we induced pyroptosis in BLaER1 and used BLaER1 GSDMD knockout cells as a control. As expected, we found BLaER1 GSDMD knockout cells to be more resistant to pyroptosis induction than BLaER1 wild type cells based on the duration until IL-1ß release and cell death. Live cell imaging revealed that induction of pyroptosis results in release of parasites from host cells and it is clearly delayed in the BLaER1 GSDMD knockout cells. Finally, we quantified the rate of parasite transfer to new host cells in a co-incubation assay. Here, the transfer rate was significantly increased in a pyroptosis dependent manner. Taken together, these findings demonstrate the suitability of BLaER1 cells as a *Leishmania* infection model to study cell to cell spread on a cell biological level and suggests pyroptosis as a cell death mechanism that can contribute to the spread of the parasites to new host cells.

A66

The novel conoidal methyltransferase PCKMT is responsible for the initiation of motility in concert with other conoidal proteins in *Toxoplasma gondii*

P. Qin[1], W. Li[1], J. Grech[1], I. Forne[2], M. Meissner[1], E. Jimenez-Ruiz[1]

[1]Ludwig-Maximilians-Universität (LMU) , Department of Veterinary Sciences, Faculty of Veterinary Medicine, Munich, Germany
[2]Ludwig-Maximilians-Universität (LMU) , Department of Molecular Biology, Faculty of Medicine, Munich, Germany

Toxoplasma gondii is an obligate intracellular parasite critically depending on invasion and egress from infected cells to ensure survival. A crucial structure for these processes is the apical complex which is highly conserved in most apicomplexans. Within this structure, the conoid, constructed by tubulin fibers, and preconoidal rings are crucial for parasite motility, invasion, and egress. Recently, we identify two proteins that localise to the preconoidal rings in two independent splitCas9 phenotypic screens: CGP and PCKMT. These proteins appear to be responsible for the initiation of motility after egress signalling. While CGP is an important structural protein for the stability of the preconoidal rings and localisation of many proteins at this structure, PCKMT, a putative lysine methyltransferase, is critical for the recruitment of formin 1 (FRM1) to the conoid. To further identify interacting proteins of CGP, FRM1 and PCKMT, we performed proximity labeling experiments using TurboID tags. These assays revealed that FRM1, CGP and PCKMT possibly interact directly with each other. Other important components for motility such as GAC and AKMT were also identified, together with additional, hypothetical identified proteins. AKMT is a methyltransferase that has been shown to affect the recruitment of GAC to the apical complex and therefore is required for motility. We hypothesise that the coordinated activation of methylation by AKMT and PCKMT is not only required for the recruitment of GAC and FRM-1 to the conoid, but a prerequisite for the initiation and maintenance of gliding motility. We currently compare the methylation of candidate proteins in presence and absence of AKMT and PCKMT respectively in an attempt to decipher the regulation of motility by methylation.

A67

Research on a child-friendly treatment against schistosomiasis, the long and winding road to develop an oral formulation of praziquantel

J. Reinhard-Rupp[1]

[1]Merck & Chair, Head, Global Health Institute, Pediatric Praziquantel Consortium, Germany

The Pediatric Praziquantel Consortium program (led by Merck) aims to close the treatment gap for preschool-aged children with schistosomiasis. Also known as bilharzia, schistosomiasis is a worm infection which affects almost 240 million people, living mainly in poor resource-setting countries in Africa and in a few countries in South America and Asia.

Since 1980, there have been tablets with the active ingredient praziquantel, which kills the adult worm. However, the disease itself can only be eliminated if the transmission cycle gets interrupted which requires treatment of all age groups. With significant international efforts and a tablet donation programme using Merck's praziquantel worth millions, the disease prevalence in many countries has been significantly reduced, but not yet eliminated.

The potential new pediatric treatment option for young children (as of the age of 6 and under) is an important milestone on the way of elimination as the target population counts an estimated 50 million preschool-aged children in need. The regulatory dossier is undergoing EMA review.

A68

Introducing the CRISPR Cas9 Cytosine Base Editor toolbox LeishBASEedit – Gene editing and high-throughput screening in *Leishmania* without requiring DNA double-strand breaks, homologous recombination or donor DNA

T. Beneke[1], *M. Engstler*[1]

[1]Universität Würzburg, Lehrstuhl für Zoologie I Zell und Entwicklungsbiologie, Würzburg, Germany

CRISPR Cas9 gene editing has revolutionized loss-of-function experiments in *Leishmania*, the causative agent of leishmanaisis. But since *Leishmania* lack a functional non-homologous DNA end joining pathway, obtaining null mutants typically requires the addition of donor DNA, selection of drug resistance-associated edits or time-consuming isolation of clones. This makes genome-wide loss-of-function screens under different conditions and across multiple *Leishmania* species unfeasible to-date. Here, we report a CRISPR Cas9 Cytosine Base Editor (CBE) toolbox to overcome these limitations. We employed CBEs in *Leishmania* to introduce STOP codons by converting cytosine into thymine and created www.leishbaseedit.net for CBE primer design in kinetoplastids. Through a series of reporter assays and by targeting single- und multi-copy genes in *L. mexicana*, *L. major, L. donovani* and *L. infantum*, we demonstrate how this tool can efficiently generate functional null mutants by expressing just one single-guide RNA, reaching up to 100% editing rate in non-clonal populations. We also generated a *Leishmania* optimised CBE version and successfully targeted an essential gene in a small-scale plasmid library delivered loss-of-function screen in *L. mexicana*. Since our method does not require DNA double-strand breaks, homologous recombination, donor DNA or isolation of clones, we believe that this enables for the first time functional genetic screens across multiple *Leishmania* species.

A69

Initiation of DNA replication in *Trypanosoma brucei* is regulated by the interaction of the RNaseH2 complex with DOT1 histone methyltransferases

L. Hartleb[1], V. Frisbie[2], H. Hashimoto[2], J. Kiselar[3], F. Butter[4], E. W. Debler[2], C. J. Janzen[1]

[1]University of Würzburg, Cell and Developmental Biology , Würzburg, Germany
[2]Thomas Jefferson University, Biochemistry and Molecular Biology, Philadelphia, PA, United States
[3]School of Medicine, Department of Nutrition, Cleveland, OH, United States
[4]Institute of Molecular Biology, Quantitative Proteomics, Mainz, Germany

Post-translational histone modifications such as lysine methylation influence the structure and the function of chromatin. DOT1 (disruptor of telomeric silencing 1) is a highly-conserved methyltransferase that exclusively methylates histone H3 lysine K79 (H3K79). *T. brucei* has two homologues, DOT1A and DOT1B, with differences in product specificity and function. While DOT1B mediates mono-, di-, and trimethylation of H3K76, DOT1A only catalyses mono- and di-methylation of this residue (H3K76me1/2). We showed previously that H3K76me1/2 is restricted to G2 phase and mitosis, and that DOT1A is a master regulator of replication initiation. RNAi-mediated depletion of DOT1A abolishes DNA replication completely whereas overexpression of DOT1A causes re-initiation of replication before the cell has completed S phase, implicating DOT1A as a master regulator of replication initiation. Since conserved regulatory domains are absent in these enzymes in *T. brucei*, the mechanism of cell cycle-dependent activity of DOT1 enzymes has remained elusive. Recently, we discovered that the ribonuclease H2 complex (RNaseH2) interacts with DOT1 enzymes in *T. brucei*. *In vitro* studies with recombinant enzymes and reconstituted nucleosomes demonstrated that RNaseH2 is an inhibitor of DOT1A activity. Using x-ray footprinting, we identified the interface region of the DOT1A/RNaseH2 complex and were able to abolish the inhibitory effect of RNaseH2 by introducing site-specific mutations into this site. Further experiments in *vivo* suggested that RNaseH2 interactions with DOT1 enzymes orchestrates their cell cycle-dependent activity in *T. brucei*, representing a novel mechanism of replication regulation.

A70

The actomyosin system of bloodstream form *Trypanosoma brucei*

S. Jung[1], F. Link[1], X. Malzer[1], M. Weiland[1], A. Konle[1], C. Batters[2], A. Borges[1], M. Pöllmann[1], J. McFarland[3], D. Nolan[3], M. Engstler[1], C. Veigel[2], B. Morriswood[1]

[1]University of Würzburg, Cell & Developmental Biology, Würzburg, Germany
[2]Ludwig-Maximilians-Universität (LMU) , Cellular Physiology, Munich, Germany
[3]Trinity College Dublin, Biochemistry & Immunology, Dublin, Ireland

The eukaryotic cytoskeleton consists of three main components: microtubules, actin filaments, and intermediate filaments. African trypanosomes (*Trypanosoma brucei)* have heavily invested in their microtubule cytoskeleton, but nonetheless retained a rudimentary actomyosin system consisting of one actin gene and two myosin genes. The exact functions of this highly reduced actomyosin system in *T. brucei* are not well understood. One of the myosins belongs to the ubiquitous class I family; the other to the trypanosomatid-specific class XXI family. The localisation of the class I myosin in the bloodstream form of *T. brucei* was refined using a combination of expansion microscopy, biochemical fractionation, and immuno-electron microscopy. Unexpectedly, around half of the myosin was cytosolic, while the remainder was strongly associated with the endolysosomal system but apparently not with the secretory pathway. The class XXI myosin was found to be expressed at a very low level, and appears to be dispensable in bloodstream form cells. Use of an anti-actin chromobody enabled the simultaneous detection of actin and myosin I for the first time in trypanosomes. Unexpectedly, there was little sign of any localisation to the flagellar pocket, suggesting a limited contribution to endocytosis. Overall, the results suggest that the actomyosin system is intimately involved in post-endocytic membrane trafficking events in *T. brucei*.

A71

Evolution of catalase in Trypanosomatidae

V. Yurchenko[1], N. Kraeva[1], Ľ. Chmelová[1], J. Sádlová[2], P. Volf[2]

[1]University of Ostrava, Life Science Research Centre, Ostrava, Czech Republic
[2]Charles University Prague, Parasitology, Prague, Czech Republic

Catalase is one of the most abundant enzymes on Earth. It decomposes hydrogen peroxide, thus protecting cells from dangerous reactive oxygen species. The catalase-encoding gene is conspicuously absent from the genome of most representatives of the family Trypanosomatidae. The exceptions are monoxenous relatives of *Leishmania* spp., and representatives of the genera *Blastocrithidia*, *Obscuromonas*, and *Vickermania.* In this work, we expressed the *Leptomonas seymouri*-derived catalase from the *Leishmania mexicana beta-tubulin* locus using a novel bicistronic expression system, which relies on the 2A peptide of *Teschovirus A*. We demonstrated that catalase-expressing parasites are severely compromised in their ability to develop in insects, to be transmitted and to infect mice, and to cause clinical manifestation in their mammalian host. Taken together, our data support the hypothesis that the presence of catalase is not compatible with the dixenous life cycle of *Leishmania*, resulting in loss of this gene from the genome during the evolution of these parasites. In addition, we ablated a catalase-encoding gene from the *Leptomonas seymouri* genome and demonstrated that parasites' development *in vivo* depends on the expression level of this enzyme. These studies were compelmented by biochemical characterization of three independently-acquired catalases (of *Blastocrithidia*, *Leptomonas*, and *Vickermania*) *in vitro.*

A72

Looking forward: Imaging the migration motor of Apicomplexa parasites

J. Periz[1], Y. Song[1], E. Jimenez[1], S. Gras[1], M. Meissner[1]

[1]Ludwig-Maximilians-Universität (LMU) , Experimental Parasitology, Munich, Germany

Apicomplexa parasites, a phylum that includes obligate intracellular pathogens such as *Plasmodium spp*, *Toxoplasma gondii* or *Cryptosporidium spp* share a repertoire of homologous structures, such as the conoid and motor proteins that are required for for motility, which is central for parasite survival and dissemination. The current view, on how Apicompexa parasites moves, is described by the linear model, that postulates that, apicomplexan parasite locomotion is a type of adhesion based motility, powered by an actin-myosin motor, located in a space of 30 nm underneath the plasma membrane, and on the outer layer of the saccule like structure called the inner membrane complex (IMC).

Although, a cumulative amount of empirical data support this model, the architecture of the motor, its actual localisation and molecular basis of migration are still largely unsolved.

Here, we systematically tagged multiple components of the gliding machinery with multifunctional reporters, such as HALO or SNAP that allow imaging of parasites using super-resolution based methods including STED, uExMIC, graphene-energy transfer (GET) biosensors and FRAP functional live imaging methods. Using this combined approach, we have started to comprehensively and objectively test whether our observations fit with the predictions from the linear model.

Our preliminary results, shows that distribution of myosin-A, actin, and other linear model components may agree with alternative configurations of the motor and alternative, mechanistic models are plausible.The data suggest a thorough revision of the current model of migration.

A73

Host immune effects on *Echinococcus multilocularis* developmental transitions

K. Brehm[1], M. Kaethner[1], K. Epping[1], K. Pätzel[1], P. Bernthaler[1], N. Leitschuh[1], I. Thomann[1], K. Rudolf[1], U. Koziol[1], M. Spiliotis[1], M. Bergmann[1]

[1]University of Würzburg, Institute of Hygiene and Microbiology, Würzburg, Germany

Introduction: The lethal zoonosis alveolar echinococcosis is caused by tumor-like growth of the metacestode larva of the tapeworm *Echinococcus multilocularis* within host organs. We previously established that the formation of the unusual metacestode is accompanied by drastic modifications of the parasite"s body axes. First, the anterior pole of the invading oncosphere is shut down within the host, resulting in the posteriorized, invasively growing metacestode. Later during an infection, the anterior pole is re-established which subsequently leads to the formation of numerous protoscoleces. How these modifications are regulated is unclear. Objectives: To investigate host influences on parasite developmental transitions and to characterize associated parasite signaling pathways. Materials & methods: We used parasite in vitro cultivation systems to study the influence of host cytokines on parasite development and differentiation, coupled with in situ hybridization and functional assays on parasite receptor systems. Results: We show that the formation of metacestode vesicles from parasite stem cells is significantly stimulated by physiological conditions of TNF-alpha, which is produced early during *Echinococcus* infections, by direct activation of a metacestode-specifically expressed TNF-alpha receptor through the host cytokine. We also show that TGF-beta, which accumulates around the metacestode during late stages of the infection, can directly stimulate *Echinococcus* TGF-receptors and induces the formation of brood capsules and protoscoleces in mature metacestode vesicles. Small molecule inhibitors against the parasite"s TGF-beta-receptors, on the other hand, blocked the formation of brood capsules and protoscoleces in otherwise unaffected, mature metacestode vesicles. Conclusions: Our data indicate that the *Echinococcus*-specific modulation of body axes which results in the formation of the metacestode is directly influenced by host cytokines that are formed during different phases of the immune response against the parasite. As such, the potentially parasitocidal, Th1-dominated immune response early during an infection fosters invasive parasite growth, whereas increased immune suppression around the parasitic lesions during late infections physiologically prepares *E. multilcularis* for the transmission to the next (definitive) host.

A74

A decade of research on neurotoxocarosis – an overview on tropisms, pathomechanisms, transcriptomics and lipidomics associated with neuroinvasive *Toxocara* larvae

M. K. Raulf[1], *A. Springer*[1], *C. Strube*[1]

[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Hannover, Germany

Neuroinvasive third-stage larvae (neural larva migrans, NLM) of *Toxocara canis* or *Toxocara cati* may lead to severe neurological symptoms in paratenic hosts, known as neurotoxocarosis (NT).

To improve the understanding of *Toxocara*-induced neuropathology, several studies focused on the investigation of NLM in the mouse as the most appropriate model organism for NT.

In the past decade, several defined aspects of NLM were unravelled such as the differential neurotropism of *T. canis* and *T. cati*, *Toxocara*-mediated neuronal demyelination, neurobehavioural as well as memory function deficits and pathological, transcriptional as well as lipidomic changes in infected mice. All of these mentioned studies were performed under similar conditions, including the same mouse and parasite strains, infective doses and examination time points, making these data suitable for comparative meta-analysis.

Initial results showed correlations between memory impairment, larval recovery, microglia activation, β-amyloid precursor protein accumulation and demyelination of infected mice. Moreover, an extended meta-analysis including differentially transcribed genes and immunomodulatory mediators will aid in elucidating whether *Toxocara*-mediated neurodegenerative processes are a cause of neurological and behavioural impairments.

In conclusion, this meta-analysis will provide a holistic and comprehensive overview on and unravel the relation between above-mentioned features of neurotoxocarosis.

A75

Ascaris suum interacts with host microbes and acquires its own microbiome

A. Midha[1], V. H. Jarquín-Díaz[2], E. Heitlinger[3], S. K. Forslund[2], S. Hartmann[1]

[1]Freie Universität Berlin, Institut für Immunologie, Berlin, Germany
[2]Max-Delbrück-Center for Molecular Medicine, Berlin, Germany
[3]Humboldt-Universität zu Berlin, Berlin, Germany

Introduction

Ascariasis is amongst the most prevalent soil-transmitted helminth infections in humans and pigs. In addition to modulating the host immune system, *Ascaris* resides in the microbial environment of the intestine and *Ascaris* infections are associated with alterations in the host microbiome. Additionally, *Ascaris* possesses its own intestine from which bacteria can be cultured. Despite the close association between intestinal nematodes and microbes, little is currently known about how nematodes contend with, or benefit from, their microbial neighbors and even less is known about the parasite"s own microbiome.

Objectives

The objectives of this work were to characterize antimicrobial strategies of helminths and to understand the elucidate the parasite microbiome and its interplay with the microbiome of its host.

Materials & Methods

Antimicrobial activities were assessed by harvesting excreted and secreted products (ESPs) of *Ascaris suum* and employing ESPs in various antimicrobial assays. Furthermore, ESPs were assessed by mass spectrometry. The *Ascaris* microbiome and the microbiome of its host were assessed by sequencing of the 16S rRNA gene in samples obtained from infected pigs.

Results

A. suum ESPs were found to possess various components with known and predicted antimicrobial activities. These products also reduced bacterial growth and disrupted biofilm formation in vitro. Furthermore, ESPs were also found to agglutinate bacteria. The *A. suum* microbiome was found to be derived, but distinct from, the microbiome of its host. In particular, dominant bacteria present at the site of infection appear to drive microbiome composition of the worm.

Conclusion

Our work presents a first look at the parasite microbiome while elucidating different antimicrobial strategies of *Ascaris* worms. These findings suggest that *A. suum* may selectively acquire microbes from their immediate surroundings.

A76

Identification of GPCR-neuropeptide interactions and their functional analyses in *Schistosoma mansoni*

X. Li[1], O. Weth[1], M. Haimann[1], S. Häberlein[1], C. G. Grevelding[1]

[1]Justus-Liebig-University Gießen, Institute for Parasitology, Gießen, Germany

Introduction

Schistosomes are parasitic flatworms that cause schistosomiasis. Standard treatment of schistosomiasis relies on a single drug, praziquantel. Neuropeptides are important messenger molecules that act via G protein-coupled receptors (GPCRs) as neurotransmitters, or hormones in the nervous system. Due to their pharmacological importance and proven druggability, GPCRs represent promising targets for new anthelmintics. Comparative transcriptomics of paired and unpaired worms and their gonads revealed 59 differentially regulated GPCR genes putatively involved in *Schistosoma mansoni* neuronal processes. Furthermore, 23 of 27 *S. mansoni* neuropeptide precursor (*Sm_npp*) genes of adult *S. mansoni* exhibited higher transcript levels in males (paired or unpaired) and unpaired females.

Objectives

Our knowledge of *S. mansoni* GPCRs and their ligands are still fragmentary. Goal of this study was to confirm rhodopsin-like orphan GPCR (*Sm*GPCR20)-*Sm_npp* interactions by biochemical methods and to characterize the appropriate partners at the molecular level including functional analyses.

Materials & methods

To characterize *Sm*GPCR20, we employed the MALAR-Y2H system. We examined the transcript profiles by quantitative real-time PCR (qRT-PCR) and localized their transcripts by whole mount *in situ* hybridization (WISH). To unravel the function of the receptor/ligand pairs, we performed RNA interference (RNAi) in adult *S. mansoni in vitro* with subsequent phenotype analysis. Confocal laser scanning microscopy (CLSM) was used for morphological analyses.

Results

We identified specific interactions between *Sm*GPCR20/*Sm*NPP26 and *Sm*GPCR20/*Sm*NPP40 and co-localized *Sm*GPCR20/*Sm*NPP26 and *Sm*GPCR20/*Sm*NPP40 expression in the head region and along the worm body in particular patterns. *Sm*GPCR20, *Sm*NPP26, and *Sm*NPP40 were found to be preferentially transcribed in bM and sM but also in sF. Phenotype analyses following RNAi against these molecules indicated a substantial decline in egg production compared with the untreated control group. Consistent with the decreased egg production, CLSM analyses revealed morphologic changes in the female gonads.

Conclusion

The obtained results suggest that *Sm*NPP26 and *Sm*NPP40 are ligands of *Sm*GPCR20, and that these molecules are involved in the molecular communication of male and female *S. mansoni* influencing egg production. Furthermore, GPCRs and neuropeptides may play important roles for male-female interaction and the control of reproduction.

A77

Age-dependent rise in IFN-γ competence undermines effective type 2 responses to intestinal nematode infection

B. Kapse[1], J. Adjah[1], H. Zhang[1], S. Hartmann[1], S. Rausch[1]

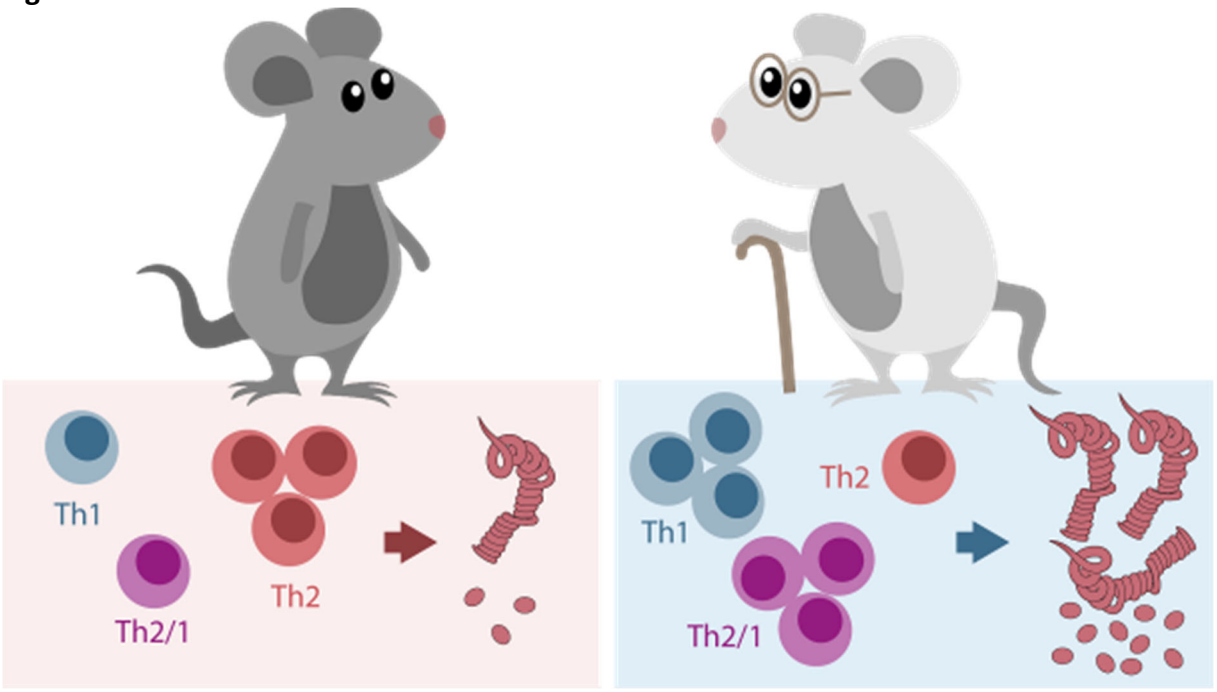
[1]Institute of Immunology, Freie Univeristät Berlin, Veterinary Medicine, Berlin, Germany

Protective type-2 immunity against helminth infection is impaired in experimental systems biased for the production of the type-1 cytokine IFN-γ. Upon birth, Th1-like memory phenotype (MP) cells generated in the absence of infection rapidly expand and accumulate over time. We hence asked whether a rise of IFN-γ competence along host age affects the development of protective type-2 responses in mice infected with the small intestinal nematode *H. polygyrus*.

Th1 cells expanded significantly in lymphoid and mucosal tissue with host age in uninfected BALB/c mice. Consequently, BALB/c mice infected at the age of 3-9 months displayed more extensive induction of IFN-γ competent, nematode-specific Th2/1 hybrid cells compared to younger animals. In more susceptible C57BL/6 mice, Th1 cells accumulated more rapidly at steady state, translating to elevated Th2/1 differentiation and poor control of parasite fitness in primary infections experienced at a young age. Blocking of IFN-γ and IL-12 signals during the first week of nematode infection leads to sharply decreased Th2/1 differentiation and promoted resistance in both mouse lines, whereas young BALB/c mice treated with IFN-γ at the onset of infection mirrored the Th2/1 hybrid-biased responses of older BALB/c as well as young C57BL/6 mice.

Together, these data show that the spontaneous accumulation of IFN-γ competent, type 1 like effector cells progressively curtails the effectiveness of anti-nematode type 2 responses with rising host age. However, elevated IFN-γ signaling early during nematode infection strongly promotes the differentiation and systemic accumulation of GATA-3+T-bet+ Th2/1 hybrid cells rather than restraining the GATA-3+ effector T cell response. In part, more extensive systemic responses in IFN-γ-rich environments could be explained by impaired colonization of effector cells in the gut alongside the restricted expansion of Foxp3+ T cells.

Fig. 1



A78

Prevalence of *Ascaris suum* and hepatitis E virus co-infections in fattening pigs in Germany

A. Laubschat[1], N. Langkabel[2], D. Meemken[2], S. Hartmann[1], J. Schlosser-Brandenburg[1]

[1]Freie Universität Berlin, Immunologie, Berlin, Germany
[2]Freie Universität Berlin, Institut für Lebensmittelsicherheit und -hygiene, Berlin, Germany

A.Laubschat1, N. Langkabel2, D. Meemken2, S. Hartmann1, J. Schlosser-Brandenburg1

1Department of Veterinary Medicine, Institute of Immunology, Centre for Infection Medicine, Freie Universität Berlin, Berlin, Germany

2Department of Veterinary Medicine, Institute of Food Safety and Food Hygiene, Section Meat Hygiene, Freie Universität Berlin, Berlin, Germany

Introduction: *Ascaris suum* is one of the most important and prevalent helminths in pigs and causes high economic losses due to weight loss in fattening pigs and liver discard at the slaughterhouse. Hepatitis E virus (HEV) is an RNA virus of the Hepeviridae family and is of considerable public health interest since domestic pigs and wild boars are the major reservoir hosts for the zoonotic genotypes HEV-3 and HEV-4. The Therefore, consumption of raw or inadequately cooked pork/liver or contact with virus-containing feces may pose a risk for human infection. Despite the frequent occurrence of both zoonotic agents in pigs, there are no studies on the prevalence of coinfection in pig herds and its impact on virus replication and shedding. Pigs have few- to no clinical symptoms which is why infections in farms remain undetected. Although both pathogens are prevalent in domestic pigs and considerable interested of public health there is no investigation yet about the effects of a Co-infection of both pathogens.

Objectives: Our hypothesis is that pig herds in which A. suum is prevalent have an increased HEV prevalence and are therefore at higher risk for zoonotic transmission. Here we assume that Th2 immunity induced by migratory worm larvae counteracts the antiviral Th1-mediated immune response in the liver. **Material and Methods:** To determine the prevalence of coinfections in German pig herds, slaughterhouse samples from fattening pigs (n=500) are serologically examined by ELISA and screened for viral RNA by qRT-PCR. Acute worm infestation of the liver is pathognomically diagnosed on the basis of milk spots. **Conclusion:** This study will provide a first insight into the prevalence of coinfection in German fattening pigs. Based on our data, new approaches could be developed to reduce HEV transmission through targeted Ascaris control strategies. This is highly relevant for the food safety of pork/liver, as well as for the exposure of veterinarians, slaughterhouse personnel and farmers.

A79

Dynamics of mucosal and systemic Th2 / Th1 effector T cells in response to tissue migrating *Ascaris suum* infections

L. Oser[1], A. Midha[1], J. Schlosser-Brandenburg[1], S. Rausch[1], R. Klopfleisch[1], S. Hartmann[1], F. Ebner[1]

[1]Freie Universität Berlin, Berlin, Germany

Introduction: The large roundworm *Ascaris suum* is one of the most relevant and frequent soil-transmitted helminths observed in pigs. Commonly, early infection and constant reinfection with *A. suum* can be observed. However, initial infection factors that may influence the development of efficient type 2 immune responses during larval migration are poorly understood. Here, we investigated the local and systemic adaptive immunity along the hepatotracheal migration route of *Ascaris suum* during primary infection in growing pigs.

Materials & methods: German-Landrace crossbreds were inoculated orally with a single dose of 4000 *A. suum* ova and dissected at different time points post before patent infection was established. Immunophenotyping for type 2 and 1 signatures was performed on PBMC and spleen and compared to local responses in nematode-migration affected tissues (liver, lung, small intestine, and their corresponding lymph nodes). In addition, *Ascaris*-specific CD4+ Th cells and their cytokine profile were assessed.

Results: Our initial data indicate that systemic Th2 levels remain constantly low during larval migration. Contrarily, we found local and transient Th2 responses in the lungs and lungLN, but not in the liver of infected animals after *A. suum* L3 had migrated through these tissues. The initial invasion of the small intestine did not elicit a type 2 response. However, Th2 cells accumulated locally with the return of larvae and their maturation into pre-adult worms. Interestingly, robust Th1 responses developed both in the circulation and in most of the investigated organs and draining lymph nodes. However, at all stages of infection, the majority of CD4+ T cells directly recognizing *A. suum* antigens produced high levels of IL-4. Meanwhile, parasite-specific IFN-g+ T cells were less frequently observed.

Conclusion: Our findings show that in growing pigs primary, experimental infection with *A. suum* does not induce a fulminant systemic type 2 response, but locally and kinetically restricted Th2 immunity. Because Th2 cells are known to orchestrate an efficient anti-parasite response, we propose that the ubiquitous and potentially infection-driven type 1 immune signature observed in conventionally raised pigs holds back the development of a strong type 2 response. Thus, further research is currently ongoing to evaluate if the weak type 2 immunity observed might contribute to susceptibility and constant reinfection observed in domestic pigs.

A80

Vector-borne parasites *Dirofilaria repens* and *Babesia canis*: haematological indices and immune response profiles in naturally infected and co-infected dogs

D. Wężyk[1], K. Romańczuk[2], A. Rodo[3], D. Kavalevich[1], A. Bajer[1]

[1]University of Warsaw, Department of Eco-Epidemiology of Parasitic Diseases, Warsaw, Poland
[2]University Warsaw, Department of Cytology, Warsaw, Poland
[3]Lab-Wet, Veterinary Diagnostic Laboratory, Warsaw, Poland

Co-infections with *Dirofilaria repens* and *Babesia canis* are rarely reported in the literature and there is very limited knowledge of their impact on canine health. Central Poland is endemic for both parasites, posing a risk of co-infections in dogs.

Our aim was to determine the effect of co-infection on canine health. We also tested the hypothesis that infection with filariae would drive the host response towards Th2-type immunity. This in turn should result in a reduction of proinflammatory Th1-type responses that are crucial for the elimination of intracellular pathogens, such as *B. canis*.

To evaluate the impact of co-infection on canine health, four groups of dogs were examined: healthy dogs, dogs infected with *B. canis*, dogs infected with *D. repens* and co-infected dogs. Blood parameters indicative of anaemia, kidney and liver damage were analysed statistically. Expression levels of immune response genes were determined and compared by qPCR reaction, in order to determine the type of immune response in mono-infection and co-infection.

In dogs infected with *D. repens*, no major alterations in blood parameters were observed. Dogs infected with *B. canis* suffered from anaemia, kidney and liver insufficiency. Co-infected dogs showed milder alternation in blood biochemical parameters associated with liver (ALP activity) and kidney (serum urea and creatinine levels) dysfunction, compared to dogs infected only with *B. canis*. The expression of genes associated with cellular (Th1-mediated) (STAT4 and INF-γ), humoral (Th2-mediated) (STAT6, GATA3, SOCS3, IL-13) and regulatory IL-10 responses was quantified. All the tested factors, except INF-γ, were found to be expressed in dogs infected with *D. repens*. The expression of IL-13 was predominant in dogs infected with *D. repens*, and the expression of STAT6 and IL-10 predominated in dogs with co-infections.

In summary, with just a few exceptions, no major alterations were found in blood parameters in *D. repens* infected dogs, and these dogs predominantly expressed Th2-related factors. While some features of Th1, T-regulatory and Th2 responses were observed in co-infected dogs, the Th2-related response appeared to predominate in these animals, and values of biochemical parameters were closer to those of healthy dogs than those solely infected with *B. canis*, suggesting a milder course of babesiosis in these animals.

The study was funded by the National Science Centre (NCN) OPUS grant no. 2017/27/B/NZ6/01691

A81

Diagnostic value of PCR-based detection of *Sarcoptes scabiei* in scabies outbreak situations

U. E. Zelck[1], N. Warncke[2], U. Karsten[2], R. Bialek[1]

[1]LADR, Molecular Microbiology, Geesthacht, Germany
[2]Gemeinschaftspraxis für Dermatologie, Geesthacht, Germany

Introduction: Scabies, a dermatological condition caused by reactions to the mite *Sarcoptes scabiei*, is an important public health issue in institutions such as nursing/ residential homes, childcare facilities, prisons and hospitals. Scabies outbreaks occurring among patients, visitors and staff are common, subject to diagnostic delay and hard to control. Verifying the clinical suspicion remains highly challenging. Time-consuming microscopic examination and dermoscopy are recommended to visualize the parasite to verify human scabies. However, microscopy has a suboptimal sensitivity, dermoscopy has low specificity and is affected by the dermatologist"s experience.

Objectives: To evaluate a new laboratory-based molecular assay as a diagnostic method for scabies, we compared PCR to microscopy and dermoscopy in a prospective study.

Patients & Methods: 163 symptomatic patients, aged 9 weeks to 99 years, and 369 asymptomatic contacts were examined. All patients underwent clinical and dermoscopic examination. To obtain skin scales, adhesive tape was applied to suspicious lesions before and after skin scraping. Tapes were mounted onto slides to enable microscopy prior to DNA extraction and *Sarcoptes scabiei* real-time PCR targeting the mitochondrial cytochrome c oxidase subunit 1 gene (cox1). Specificity of the PCR assay was verified by using DNA extracted from other common mite species.

Results: Mite or mite products were detected by microscopy in only 8% of the patients. By dermoscopy, mites were detected in 88% (95% CI: 82·3 – 93·0) and *Sarcoptes scabiei* DNA was detected by PCR in 87% of patients (95% CI: 80·0 – 91·4). No cross-reactivity to other relevant mites was detected. Scabies PCR offers an improvement in sensitivity to verify scabies compared to microscopy and performed equivalently to dermoscopy (p-value 0·628). Skin scraping before applying adhesive tape significantly increased success (87% vs 56%; p <0·001).

Conclusion: Our PCR-based method from skin scrapings is nontraumatic, non-expert-dependent and reproducible. *S. scabiei* PCR is specific, sensitive and as successful as dermoscopy, but requires no specific equipment or expertise on-site. This PCR-based diagnostic method has the potential to accelerate diagnosis and treatment, ultimately limiting or preventing scabies outbreaks in care homes or other close communal environments. PCR-based verification can also be used as an objective diagnostic criterion for scabies in therapeutic trials.

A82

Sero-diagnosis of visceral leishmaniasis in East Africa and development of a new therapy by testing the *Leishmania* proteasome as target molecule

U. Steinhoff[1], *R. Mahdavi*[1]

[1]Philipps-University Marburg , Medical Microbiology and Hospital Hygiene, Marburg, Germany

Reliable and field suitable sero-diagnosis of visceral Leishmaniasis (VL) in East Africa is till today a big challenge due to low sensitivity and crossreactivity with other pathogens. To improve VL sero-diagnosis, new and field suitable POC tests were developed on the basis of a recombinant kinesin antigen from *L. infantum* (rKLi8.3). The new rkLi8.3 based sero-diagnostic devices revealed improved sensitivity and no cross-reactivity when tested with sera from patients originating from Sudan, India and South America, suffering from VL, malaria, tuberculosis and trypanosomiasis. We could show that rKLi8.3 based ELISA and LFT offer substantially increased diagnostic efficiency for VL in East Africa and other endemic areas, compared to currently commercial available sero-diagnostic tests.

Besides diagnosis, the current treatment of visceral leishmaniasis is also far from optimal, as many drugs induce resistent Leishmania strains and have severe side effects. It has been shown that the family of kinetoplastida *(T. brucei*, *T. cruzi* and Leishmania spp.) are highly susceptible to the proteasome inhibitor, GNF-6702. Characteristic sequence variations within the proteasome beta 4 and beta 5 subunits of kinetoplastida have been made responsible for the specific binding of GNF-6702 to the proteasome of kinetoplastia but not humans. To test whether these proteasome subunits are conserved throughout various Leishmania isolates and differ from the human proteasome, we analyzed the beta 4 and beta 5 proteasome subunits of 15 different Leishmania strains and found conserved AA substitutions in the beta 4 proteasome subunit of all strains analayzed, indicating that GNF-6702 is a potential drug for VL. Further, analyzing the parasite survival by treating pro-and amastigote *L. donovani* with inhibitors for constitutive - (GNF-6702) or immuno-proteasomes (ONX-0914) revealed that ONX-0914 had no effect on promastigote parasites but strong effects on amastigotes. This finding strongly suggests that the pressure of the intracellular lifestyle alters the proteasome composition from a constitutive to an immuno-like form. We are currently characterizing the proteasome subunit composition of pro-and amastigote Leishmania.

A83

Current progress in reverse vaccinology towards *in silico* vaccine discovery for parasitic diseases

S. Goodswen[1], P. Kennedy[2], J. Ellis[1]

[1]University of Technology Sydney, Life Sciences, Ultimo, Australia
[2]University of Technology Sydney, Computer Science, Ultimo, Australia

Introduction

Reverse vaccinology (RV) was first described in 2000 as an *in silico* process that starts from the genomic sequence of the pathogen and ends with a list of potential vaccine candidates. Subsequently, other *in silico* related processes with overlapping workflows have also emerged with terms such as subtractive proteomics, computational vaccinology, and immunoinformatics. From the perspective of a new RV practitioner, determining the appropriate workflow steps and bioinformatics tools is a time consuming and overwhelming task.

Objectives

We document the current knowledge on RV and its usage in the research community by a comprehensive survey of scientific papers published with "reverse vaccinology" (RV) in the title from 2015 to 2021.

Materials & methods

All papers published with "reverse vaccinology" in their title were manually reviewed (140 papers in total, source: Web of Science). There were, however, 205 additional papers from the same period with RV in the abstract but not in the title. We made no attempt to capture current RV status in an unknown number of papers using an *in silico* vaccine discovery approach but with no reference to RV in the title or abstract.

Results

287 different bioinformatics tools were used in one or more of the RV workflows and some are more popular than others. 95.6% of the workflows rely on online tools. The RV pipelines developed so far mainly predict proteins naturally exposed to the immune system.

The workflow for selecting candidates in 87.8% of the latest publications involves a consecutive filtering process and not machine learning (ML). To automate the RV process, software pipelines were developed and made freely available since 2006. There are currently 11 known RV-related pipelines and almost all of them perform candidate ranking by ML for this purpose. They are not frequently used.

Currently, only 12.2% of the latest publications report tests on animal models providing a measure of success on whether an RV-derived candidate induces a protective response.

Conclusion

The majority of surveyed publications use classical RV as only one stage in a broader series of workflow stages to computationally identify vaccine candidates. Installation of a standalone program and/or adapt an API is a major disincentive to RV practitioners. We recommend "*in silico* vaccine discovery" should be consistently used in titles, abstracts, and/or keywords of future publications to unify the discipline area of RV.

A84

Use of species-customized Rat Basophilic Leukemia (RBL) IgE reporters for diagnosis of *Echinococcus granulosus* infection and *Taenia solium* cysticercosis

P. S. Prakash[1], M. H. W. Weber[1], M. S. Hossain[1], S. Shabir[1], P. Toye[2], L. F. Thomas[2], J. J. van Hellemond[3], F. H. Falcone[1]

[1]Justus-Liebig-University Gießen, Institute for Parasitology, Gießen, Germany
[2]International Livestock Research Institute (ILRI), Nairobi, Kenya
[3]Erasmus MC University Medical Center, Department of Medical Microbiology and Infectious Diseases, Rotterdam, Netherlands

Echinococcus granulosus is the causative agent of cystic echinococcosis in humans infected as accidental hosts. Similarly, cysticercosis is caused by accidental ingestion of *Taenia solium* eggs by pigs and humans. Diagnosis is usually achieved using computerized imaging technologies, complemented by serological analyses. However, the latter have serious limitations in terms of specificity and, to a lesser extent, sensitivity. Even though *Echinococcus* infection induces a strong IgE response in infected hosts including human, this isotype is currently underexploited by current serological diagnostic techniques, which are mostly based on detection of parasite-specific IgG.

Here, we show proof-of-principle that humanized IgE reporter systems can be used advantageously for diagnosis of cystic echinococcosis. We introduce our new RBL NPY-mRFP reporter system, which requires neither expensive substrates nor overnight incubation for detection of activation. We are also adapting the reporter systems for detection of IgE in dogs and pigs.

Our data obtained using raw cyst fluid as antigen demonstrate the high discriminating power of IgE-based reporter systems for cystic echinococcosis diagnosis. We are currently employing several immunological and bioinformatic techniques, together with recombinant expression and protein purification, to identify known and novel *Echinococcus granulosus* and *Taenia solium* allergens in cyst fluid and oncospheres and assessing their suitability as diagnostic antigens using our reporter systems.

A85

Physiological jump in erythrocyte redox potential during *Plasmodium falciparum* development occurs independent of the sickle cell trait

M. Haag[1], J. Kehrer[1], C. Sanchez[1], M. Deponte[2,1], M. Lanzer[1]

[1]RKU Heidelberg, Heidelberg University Medical School, Heidelberg, Germany
[2]TU Kaiserslautern, Chemistry/Biochemistry, Kaiserslautern, Germany

1. Introduction

The redox state of the host-parasite unit has been hypothesized to play a central role for the fitness of the intraerythrocytic blood stages of the human malaria parasite *Plasmodium falciparum*. In particular, hemoglobinopathies have been suggested to cause a more oxidizing environment, thereby protecting from severe malaria.

2. Objectives

We wanted to determine the redox state of the host-parasite unit during *P. falciparum* blood-stage development in wild-type (hemoglobin AA) or sickle trait (hemoglobin AS) erythrocytes.

3. Materials & Methods

We used parasite-encoded targeted variants of the redox-sensitive green-fluorescent protein 2 (roGFP2) for stage-dependent confocal microscopy redox measurements in wild-type or sickle trait erythrocytes.

4. Results

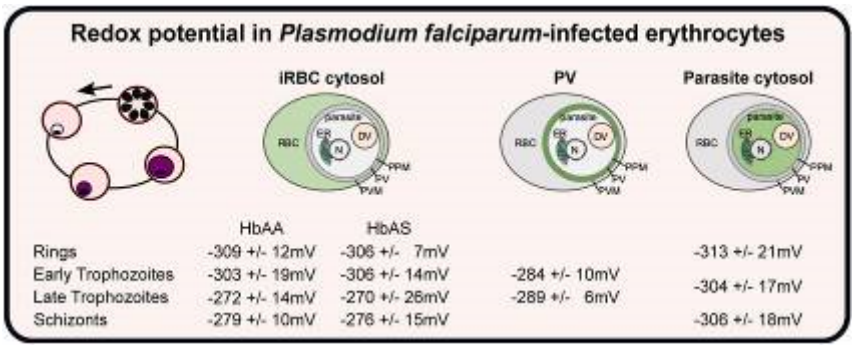
Our roGFP2 single-cell measurements revealed a reducing steady-state redox potential of −304 ± 11 mV for the erythrocyte cytosol during ring-stage development and a rather sudden oxidation to −278 ± 12 mV during trophozoite-stage development around 28 h post invasion. There was no significant difference between wild-type or sickle trait erythrocytes regarding the stage dependence and the detected increase of the redox potential during the intraerythrocytic life cycle. The steady-state redox potential of the parasite cytosol, between −304 and −313 mV, was highly reducing throughout the life cycle. The redox potential in the parasitophorous vacuole at the interface between the secretory pathway and the erythrocyte was −284 ± 10 mV and remained stable during trophozoite-stage development with implications for the export of disulfide-containing proteins.

5. Conclusion

Non-invasive roGFP2 measurements revealed a much more reducing steady-state redox potential of the erythrocyte and parasite cytosol than deduced from invasive glutathione measurements. Parasite-dependent modifications of its host cell result in a physiological +30 mV jump in erythrocyte redox potential during trophozoite-stage development that can be explained by a drastic efflux of reduced glutathione. Most important, there was no difference between the stage-dependent steady-state redox potentials of infected wild-type or sickle trait erythrocytes in contrast to the often iterated hypothesis that so-called oxidative stress protects from malaria [1].

[1] Haag M et al. 2022 Redox Biol 58: 102536.

Fig. 1


Redox potential in *Plasmodium falciparum*-infected erythrocytes
iRBC cytosol
PV
Parasite cytosol
RBC
ER
N
DV
PPM
PV
PVM
HbAA
HbAS
Rings -309 +/- 12mV -306 +/- 7mV -313 +/- 21mV
Early Trophozoites -303 +/- 19mV -306 +/- 14mV -284 +/- 10mV -304 +/- 17mV
Late Trophozoites -272 +/- 14mV -270 +/- 26mV -289 +/- 6mV
Schizonts -279 +/- 10mV -276 +/- 15mV -306 +/- 18mV

A86

Role of endothelial cells and miRNA in the pathogenesis of human malaria caused by *Plasmodium falciparum* infection

Y. Wu[1], S. Leyk[2], H. Torabi[1], K. Höhn[3], B. Honecker[4], M. D. P. Martinez Tauler[4], D. Cadar[5], T. Jacobs[2], I. Bruchhaus[4], N. G. Metwally[1]

[1]Bernhard Nocht Institute for Tropical Medicine, RG Host Parasite Interaction, Hamburg, Germany
[2]Bernhard Nocht Institute for Tropical Medicine, RG Protozoa Immunology, Hamburg, Germany
[3]Bernhard Nocht Institute for Tropical Medicine, Cellular Parasitology Department, Hamburg, Germany
[4]Bernhard Nocht Institute for Tropical Medicine, RG Host Parasite Interaction, Hamburg, Germany
[5]Bernhard Nocht Institute for Tropical Medicine, Arbovirology Department, Hamburg, Germany

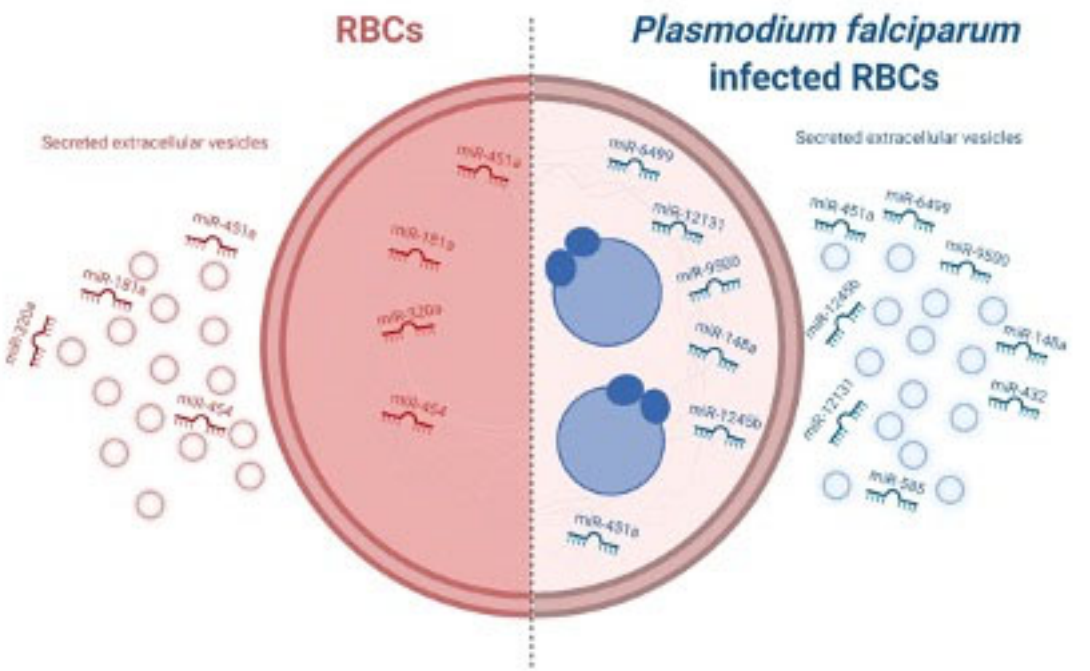
Plasmodium falciparum is known to cause severe complications to the human host due to cytoadhesion of infected erythrocytes (IEs) to host endothelial cells (ECs). In recent years, evidence has accumulated that cytoadhesion is not the only explanation for the complications associated with malaria. Indirect cell-cell communication can occur through secretion of extracellular vesicles (EVs) into the circulation, containing proteins, DNA, mRNA and microRNAs (miRNAs). Secreted EVs can be taken up by recipient cells, and EVs miRNAs can alter the gene expression in these cells. EVs derived from IEs are known to contain both parasite proteins and human miRNAs. miRNAs can block mRNA by directing degradation through a protein complex or by destabilization of the messenger. Altered miRNA profiles have been described for many types of infections. The miRNA profiles of human ECs during malaria pathogenesis have not yet been studied. We postulate that dysfunction of ECs may play an important role in the prognosis of severe malaria complications. Certain miRNA candidates might control these events by affecting gene expression within the ECs.

Methods: In this study, we investigated if miRNAs play a role in pathogenesis during *P. falciparum* infection. First, we analysed the miRNA expression profile in the IEs as well as their secreted vesicles using NGS sequencing. Second, we characterised the mRNA and miRNA expression profiles of human brain ECs exposed to co-incubation with *P. falciparum* IEs.

Results: NGS and bioinformatic analysis showed that 20 miRNA candidates were differentially expressed in the vesicles secreted from the IEs compared to the non-infected ones. In addition, 8 mature microRNA candidates were differentially downregulated in the vesicles secreted from the IEs compared to the non-infected ones. Upon co-incubation of human brain ECs with *P. falciparum* under low shear stress 8 mature microRNA candidates were differentially expressed.

Conclusion: Our experiments showed that the microRNA profiles of the human erythrocytes and ECs are affected during *P. falciparum* infection. Mapping these changes might give us more clues about the inflammatory pathways that are turned on during the severe outcomes of the infection.

Fig. 1


RBCs
Plasmodium falciparum infected RBCs
Secreted extracellular vesicles
Secreted extracellular vesicles
miR-451a
miR-181a
miR-1260
miR-454
miR-6499
miR-12131
miR-9500
miR-146a
miR-12456
miR-451a
miR-451a
miR-181a
miR-1260
miR-454
miR-6499
miR-9500
miR-12456
miR-146a
miR-12131
miR-432
miR-585

A87

Human brain endothelial cell activation during *Plasmodium falciparum* infection

Y. Wu[1], H. Torabi[1], S. Lorenzen[1], I. Bruchhaus[1], N. G. Metwally[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Introduction:

Sequestration of ***Plasmodium falciparum*** in human microvessels occurs due to an interaction between a parasite ligand and host endothelial cell (EC) receptor in a process called cytoadhesion. Cytoadhesion results in microvascular obstruction, impaired perfusion, hypoxia and finally organ failure. ***Pf***EMP1 (***Plasmodium falciparum*** erythrocyte membrane protein-1) is encoded by approximately 60 ***var*** genes per parasite genome and is considered the most important surface antigen of ***P. falciparum***-infected erythrocytes (IEs).

Objectives:

Characterization of the tropism of *Pf*EMP1 for different EC receptors and the subsequent immune response triggered by cytoadhesion.

Materials & methods:

In this study, we enriched *P. falciparum* isolates IT4 over resting or TNF-α-activated human brain microvascular endothelial cells (HBMECs). Furthermore, we stimulated HBMECs with enriched parasites for 4 hours and 8 hours separately and characterized the HBMECs transcriptomic changes. Moreover, in order to simulate the ***in vivo*** condition we stimulated HBMECs with IEs under defined shear stress. mRNA and miRNA profiles of stimulated HBMECs were characterized by NGS.

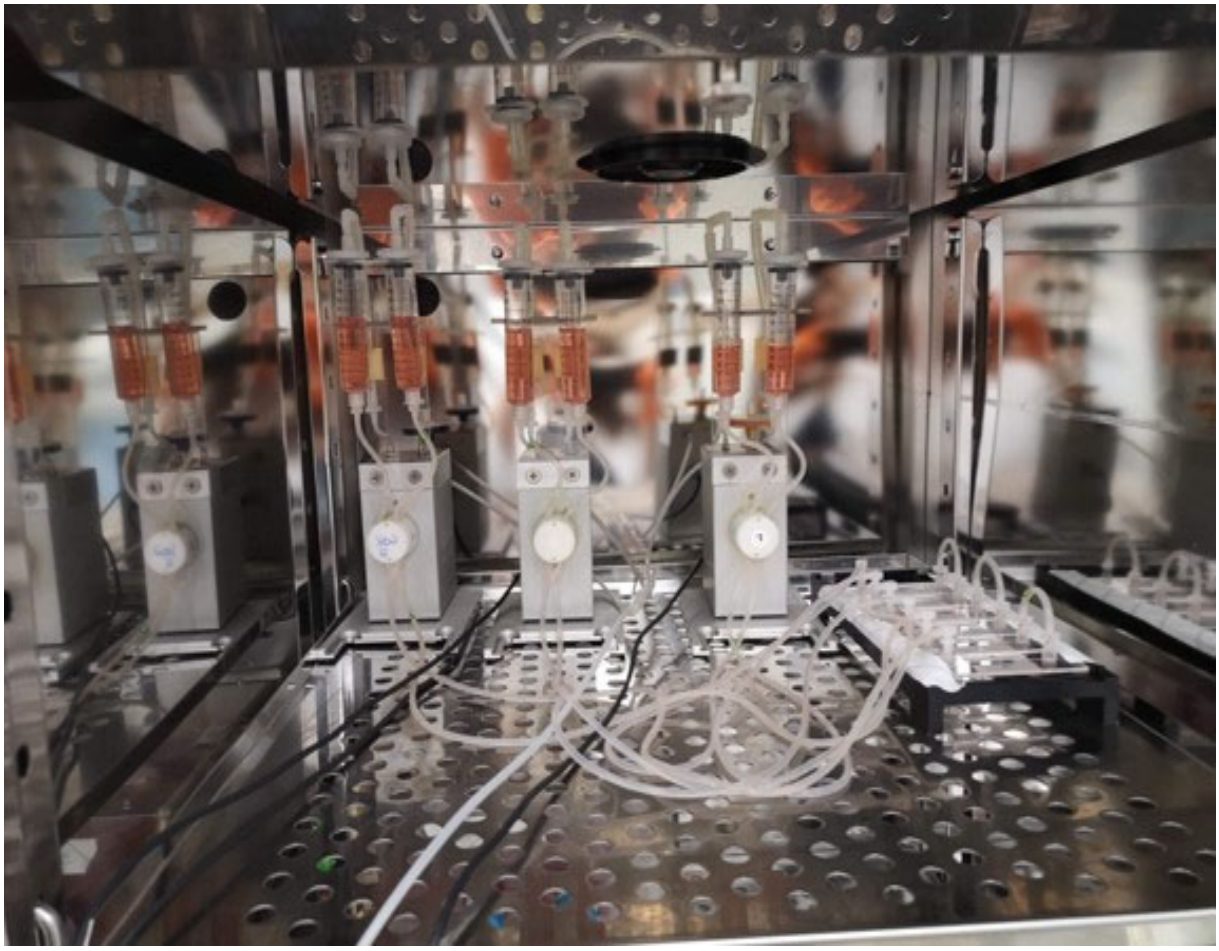
Results:

Transcriptome analysis indicated that 101 differentially expressed genes (DEGs) were identified in HBMECs after 4 hours of stimulation and 51 DEGs were identified in HBMECs after 8 hours of stimulation. Reactome analysis of DEGs revealed the enrichment in antigen presentation and interferon gamma signal pathway. Interestingly, we found the mRNA expression level of oligoadenylate synthase (OAS) gene family increased significantly after 4 hours of stimulation.

Conclusion:

By comparing the transcriptomes of HBMECs stimulated for 4 h and 8 h, we observed upregulation of immunoregulatory genes within the first 4 h of stimulation. However, these immune response declines by reaching 8 h of coincubation. Besides, shear stress stimulation may affect the mRNA and miRNA profiles of HBMECs.

Fig. 1



A88

Reverse genetics reveals new genes involved in host cell modification by the malaria parasite *P. falciparum*

L. Roling[1], N. Küster[1], A. Ouayoue[1], J. M. Przyborski[1]

[1]Justus-Liebig-University Gießen, Biochemistry and Molecular Biology of Infection, Gießen, Germany

Following invasion of human erythroyctes, the malaria parasite *P. falciaprum* massively changes the biochemical and biophysical properties of the host cell. This process directly and indirectly underpins the pathology associated with malaria infection, and is driven by a large number of proteins encoded by the parasite, and which are transported to the host cell. To understand to role specific exported proteins play in host cell modification, we initiated a medium-scale reverse genetic analysis of a number of genes of interest (GOI) we believed may be involved in this process. Over the course of 6 years we generated 76 genetically modified parasite lines using a number of techniques. In contrast to previous studies, we find that a large majority of the GOI (14/18) are resistant to both direct inactivation, or introduction of the GFP coding sequence. Use of a smaller tag, 3xHA, allowed us to generate 5 further parasite lines which also included a *glmS* ribozyme to allow modulation of protein levels. Additionally, we obtained 2 knockout lines. A detailed analysis of three parasite lines revealed involvement of the GOI/POI in a number of host cell modification processes and uncovered novel mutant phenotypes never before reported. Further analysis of one specific parasite line lacking an exported J-domain protein highlighted a potential role for residual human HSP70 proteins in host cell modification processes, the first time this has been experimentally demonstrated. Taken together, our data highlight the importance of exported proteins for parasite growth, survival and pathology in the human host and host cell, and underpin the complex nature of the host parasite interaction at a molecular level.

A89

The role of host Rab GTPases during the intracellular liver stage of *Plasmodium berghei*

O. Netzel[1], *A. Ingmundson*[1]

[1]Humboldt University of Berlin, Molecular Parasitology , Berlin, Germany

1. Introduction

The liver stage of Plasmodium berghei is marked by extensive growth of a few sporozoites to produce thousands of progeny and establish the blood-stage infection. To ensure the fast growth, the parasite needs to acquire nutrients from within the host cells. Although late endosomes and the Golgi apparatus have been shown to associate with the parasitophorous vacuole in which the parasite resides, we hypothesize that additional host membrane compartments contribute to a successful liver stage infection.

2. Objectives

In our study, we wanted to identify membrane trafficking pathways that interact with the parasitophorous vacuole. To this end, we investigated the localization of host Rab GTPases during the infection. Rab GTPases are key regulators of specific trafficking events, which might be altered by the parasite. Other intracellular parasites have been shown to target specific Rab proteins to ensure their survival.

3. Materials & Methods

We infected hepatoma cells expressing tagged Rab GTPases and infected them with P. berghei. Through microscopy, we observed the localization of the different Rab proteins in infected cells at various time points during the infection. With additional immunofluorescence staining we determined the arrangement of additional host and parasite structures.

4. Results

Our initial screening of different Rab proteins showed association of Rab-labelled membranes with the parasite. Amongst predicted candidates like late endosomes, additional specific membrane compartments were observed accumulating in close proximity to the parasitophorous vacuole. Intriguingly, we identified a host Rab protein that not only accumulates near the parasite but is present on the parasitophorous vacuole. It showed clear colocalization with the parasitophorous vacuole membrane marker UIS4 throughout the development of the parasite.

5. Conclusions

Our data demonstrate that specific host membranes can be found in close proximity to the parasite-containing vacuole during infection. The presence of a host Rab on the membrane surrounding the parasite suggests this particular candidate is relevant to the infection. The localization of this Rab and of the neighboring host vesicles suggests some interplay between host Rab GTPases and the growing parasites.

A90

Contribution of Actin-like proteins Alp1 and Alp2b in *Plasmodium* progression

Y. Kobayashi[1], A. Binder[2], F. Mikus[2], F. Frischknecht[2], R. Douglas[1]

[1]Justus-Liebig-University Gießen, Molecular Infection Biology, Gießen, Germany
[2]Heidelberg University Medical School, Integrative Parasitology, Heidelberg, Germany

Plasmodium requires active motility in order to spread and must traverse and invade mammalian and mosquito organs. Actin-like proteins (Alps) are apicomplexan-specific, highly divergent members of the actin superfamily. Alps are characterised by several unique regions and likely have critical functions during parasite infection. However, the functions and stage specific contributions of Alps in parasite progression are unknown.

The corresponding genes of *Alp1* and *Alp2b* were knocked-out in *Plasmodium berghei* and the phenotypic impacts of these deletions on the parasites were investigated. Remarkably, the absence of Alp1 or Alp2b led to a drastic reduction or complete block of mosquito midgut colonisation at different steps of transmission respectively. Specifically, Alp1 was essential for the motility of ookinetes, which actively colonise mosquito midguts, while Alp2b was crucial for parasite fertilisation. Alp2b function in *P. berghei* was partially restored by *P. falciparum Alp2b* complementation, indicating a degree of cross-species conservation. Mutations of selected unique regions of *P. berghei* Alp2b prevented exflagellation, implicating these regions as critical contributors to Alp2b function and thus potential targeting regions.

Our study reveals the importance of Alps in *Plasmodium* transmission and the stage-specific contributions of these members of apicomplexan actin superfamily.

A91

Parasite G6PD is a promising target for drug development against *Leishmania*

K. Bagola[1], M. Bergmann[1], S. M. Schmelzle[1], I. Berneburg[2], B. Walber[1], S. Rahlfs[2], J. M. Przyborski[2], P. Kolb[3], G. van Zandbergen[1]

[1]Paul-Ehrlich-Institut, Immunology, Langen, Germany
[2]Justus-Liebig-University Gießen, Biochemistry, Gießen, Germany
[3]Philipps-University Marburg , Pharmaceutical Chemistry, Marburg, Germany

The enzyme glucose-6-phoshate dehydrogenase (G6PD) of the pentose phosphate pathway is required for redox regulation. As G6PD is found amongst numerous parasites causing neglected tropical diseases (NTD), including *Leishmania (L.)*, identifying a drug to target G6PD in various *Leishmania* strains is a very promising strategy.

In order to genetically validate *Leishmania* G6PD as a potential drug target, we employed CRISPR/Cas9 to knockout this gene in both cutaneous and visceral Leishmaniasis (CL and VL) causing strains. Knock out of both alleles appeared to be lethal, viable parasites were only obtained while expressing *Leishmania* G6PD from a rescue plasmid. Loss of this plasmid resulted in parasite death, pointing to an essential role of G6PD for parasite survival. To further evaluate the role of G6PD in *Leishmania,* we established a CRISPR/Cas9/DiCre inducible knockout system. In this system parasites loose G6PD gene expression as well as G6PD enzymatic activity, over a time period of seve days resulting in both *L. donovani* and *L. major* full G6PD knockout strains. Interestingly wild type *L. donovani* contained a much higher level of G6PD activity as compared to wild type *L. major* parasites. By flow cytometry analysis, we could show that G6PD knockout decreases the parasites" tolerance against reactive oxygen species in the promastigote life stage of both species. In contrast to the viable *L. major* G6PD deficient axenic amastigotes, the *L. donovani* G6PD induced knockout strain was unable to survive as axenic amastigotes. Moreover preliminary data suggest that the *L .major* G6PD knockout had does not survive inside human macrophages. In summary, our data genetically validate G6PD as a promising drug target in both CL and VL causing *Leishmania,* and support the strategy to develop drugs for targeting multiple parasites causing NTDs.

A92

The *Fasciola* $TRPM_{PZQ}$ channel, a new druggable target

L. Bauer[1], D. J. Sprague[2], S. Gramberg[1], J. S. Marchant[2], S. Häberlein[1]

[1]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany
[2]Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI, United States

Introduction

Milestones have recently been achieved in clarifying the mechanism of action of praziquantel (PZQ), the drug of choice to fight schistosomes. PZQ acts as an agonist of a transient receptor potential (TRP) channel within the melastatin family ($SmTRPM_{PZQ}$), which leads to rapid contraction of the worms and death. Mutagenesis of residues within the binding pocket of $SmTRPM_{PZQ}$ identified an asparagine (Asn) residue as crucial factor for PZQ-sensitivity. While the TRPM channel of other PZQ-sensitive helminths harbor the same Asn, natural variation at this residue renders $TRPM_{PZQ}$ of the liver fluke *Fasciola hepatica* ($FhTRPM_{PZQ}$) resistant to PZQ (Park et al. 2021). Exploring $FhTRPM_{PZQ}$ as drug target opens new avenues to find new fasciolicidal compounds. This is particularly pressing against the wide spread of triclabendazole resistance.

Objectives

In this study, we aim for a *de novo* discovery of agonists for the *F. hepatica* $TRPM_{PZQ}$ channel and to test if such agonists have fasciolicidal activity.

Materials and Methods

A high throughput drug screen using a Ca^{2+}-based reporter assay (Chulkov et al. 2021) was used to identify $FhTRPM_{PZQ}$-activating chemotypes. One of these chemotypes was tested against $FhTRPM_{PZQ}$ and different stages of *F. hepatica in vitro*. Further characterization of $FhTRPM_{PZQ}$ is envisaged by RNA interference and gene expression analysis.

Results

The reporter assay identified several chemotypes triggering Ca^{2+} influx in $FhTRPM_{PZQ}$-transfected cells. One series was selected and optimized to yield a ligand that activated $FhTRPM_{PZQ}$ with high sensitivity *in vitro* (EC50=855±151nM). The same chemotype killed adult and immature *F. hepatica* at concentrations of 6.25-12.5 µM within 24 h-72 h. The TRPM agonists, therefore, outcompeted triclabendazole, which was lethal at 50 µM within 48 h. Contraction of the worms resembled the phenotype observed for PZQ-treated schistosomes. Finally, our single-cell transcriptomics data suggest a neuronal expression of FhTRPM.

Conclusion

Our study proves that $TRPM_{PZQ}$ is a druggable target also in a PZQ-resistant helminth. The successful screening approach may be translated to other PZQ-resistant helminths in the future.

Park et al. Mechanism of praziquantel action at a parasitic flatworm ion channel. Sci Transl Med. 2021;13(625):eabj5832.

Chulkov et al. Identification of novel modulators of a schistosome transient receptor potential channel targeted by praziquantel. PLoS Negl Trop Dis. 2021;15(11):e0009898.

A94

A synergistic approach identifies key host factors required for apicomplexan parasites

M. Maurizio[1], K. Woods[1], R. Caldelari[2], M. Masid[3,4,5], J. Doench[6], J. Zemp[1,2], A. Naguleswaran[1], V. Hatzimanikatis[3], V. Heussler[2], S. Rottenberg[1], P. Olias[1,7]

[1]University of Bern, Vetsuisse Faculty, Bern, Switzerland
[2]University of Bern, Institute of Cell Biology, Bern, Switzerland
[3]École Polytechnique Fédérale de Lausanne, Laboratory of Computational Systems Biotechnology, Lausanne, Switzerland
[4]University of Lausanne, Department of Oncology, Lausanne, Switzerland
[5]Lausanne University Teaching Hospital, Lausanne, Switzerland
[6]Broad Institute of MIT and Harvard, Cambridge, MA, United States
[7]Justus-Liebig-University Gießen, Institute of Veterinary Pathology, Gießen, Germany

Apicomplexan parasites are obligate intracellular organisms responsible for major human and bovine infectious diseases, including malaria, toxoplasmosis and theileriosis. In this project we used a synergistic approach to identify host cell pathways that are essential for the parasite but dispensable for the host cell. By performing a genome-wide CRISPR/Cas9 drop out screen in *Theileria*-infected and non-infected bovine macrophages we obtained a list of 111 genes specifically essential for infected cells, with one third of these involved in metabolic pathways. We then combined genetic screens with chemoinformatic modelling, and ran gene knock-out simulations of these candidates in a human hepatocyte-*Plasmodium falciparum* metabolic model. Hydroxymethylbilane synthase (HMBS), an enzyme involved in heme biosynthesis, was predicted to be dispensable for uninfected hepatocytes but necessary for the survival of *Plasmodium* in the host cell. In order to confirm this prediction, we generated HMBS knockout clones in the human haploid cell line HAP1. Upon infection with the widely used rodent model organism *Plasmodium berghei*, we observed a significant decrease in parasite numbers, accompanied by a striking reduction in parasite liberation from infected cells. To exclude Cas9 off-target effects, we complemented HMBS and obtained a complete reversion of the phenotype. In conclusion, our results indicate that both *Plasmodium* and *Theileria* parasites depend on host HMBS for their survival. This suggests that *Plasmodium*, which expresses all enzymes of the heme pathway, not only relies on endogenous heme biosynthesis but also requires an intact host pathway for liver stage development.

A95

IFN lambda protects mice from lethal oral *T. gondii* infection

T. Steinfeldt[1], M. Murillo-Léon[1], A. M. Bastidas-Quintero[1], N. S. Endres[2], D. Schnepf[3], E. Delgado Betancourt[4], A. Ohnemus[1], G. A. Taylor[5], M. Schwemmle[1], P. Staeheli[1]

[1]University Medical Center, Department of Medical Microbiology, Virology and Hygiene, Freiburg i. Br., Germany
[2]Charité – Universitätsmedizin Berlin, Institute of Virology, Berlin, Germany
[3]The Francis Crick Institute, Immunoregulation Laboratory, London, United Kingdom
[4]Robert-Koch-Institute, FG 16: Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany
[5]Duke University Medical Center, Departments of Medicine; Molecular Genetics and Microbiology; and Immunology, Durham, NC, United States

Toxoplasma gondii (*T. gondii*) is food-borne parasite that is an important cause of clinical disability in humans. Bradyzoites or sporozoites released upon rupture of tissue cysts or oocysts disseminate throughout the host. Interferons are essential for innate and adaptive immune responses against a wide variety of pathogens. Interferon lambda (IFN-λ) protects mucosal barriers during pathogen exposure. The intestinal epithelium is the first contact site for *T. gondii* with its hosts and the first defense line that limits parasite infection. Knowledge of very early *T. gondii* infection events in the gut tissue is limited and a possible contribution of IFN-λ has not been - to the best of our knowledge - investigated so far. Here, we demonstrate with systemic interferon lambda receptor (IFNLR1) and conditional (Villin-Cre) knockout (ko) mouse models of oral *T. gondii* infection and mouse intestinal organoids a significant impact of IFN-λ signalling in intestinal epithelial cells. Furthermore, a probe-based qPCR reveals increased parasite load in the ileum in ko compared with wt animals. Infective parasite stages cross the intestinal epithelial barrier to infect the lamina propria. In the lamina propria, *T. gondii* replicates in a variety of different cell types before disseminating throughout the host. Microsections of intestinal tissue demonstrate augmented *T. gondii* replication in the lamina propria of ko compared with wt animals. Besides intestinal epithelial cells, our results obtained with bone marrow-chimeras reveal an additional impact of IFN-λ signalling in neutrophils to parasite control. This study expands the repertoire of interferons that contribute to the control of *T. gondii* in mice and may lead to novel therapeutic approaches against this world-wide zoonotic pathogen.

A96

Experimental infection of sheep at mid-pregnancy with archetypal type II and type III *Toxoplasma gondii* isolates exhibited different phenotypic traits

R. Vallejo[1], J. Benavides[2], N. Arteche-Villasol[1], R. Sánchez-Sánchez[3], R. Calero-Bernal[3], M. d. C. Ferreras[1], V. Pérez[1], L. M. Ortega-Mora[3], D. Gutiérrez-Expósito[1]

[1]University of León, Spain, Animal Health, León, Spain
[2]Instituto de Ganadería de Montaña, Animal Health, León, Spain
[3]Complutense University of Madrid, Animal Health, Madrid, Spain

Introduction

The genotypic diversity of *Toxoplasma gondii* has been associated with variations in phenotypic traits in *in vitro* and murine models. However, whether such diversity could influence the outcome of infection in small ruminants remains mostly unexplored

Objectives

To investigate the outcome of oral challenge in sheep at mid-pregnancy with 10 sporulated oocysts from three different *T. gondii* isolates belonging to archetypal II and III and selected according to their genetic and phenotypic variations shown in previous studies

Material & methods

Seventy-three pregnant sheep were divided in four groups: G1 infected with TgShSp1 isolate (type II, ToxoDB#3), G2 with TgShSp16 isolate (type II, ToxoDB#3), G3 with TgShSp24 isolate (type III, ToxoDB#2) and G4 of uninfected control sheep. Two different approaches were carried out within this study: (i) the outcome for the pregnancy after infection (*n*=33) and (ii) the lesions and parasite tropism and burden at 14- and 28-days post infection (dpi) (*n*=40)

Results

The onset of hyperthermia and seroconversion occurred one and two days later, respectively in G1 when compared to G2 and G3. However, sheep that suffered from reproductive failure, either by abortion, fetal dead at the time of euthanasia or stillbirth were similar among infected groups (50%, 40% and 47%, respectively). Histological lesions in placentomes and fetal tissues from euthanized animals from the second approach were only detected at 28 dpi and mainly in G1. At 14 dpi, *T. gondii*-DNA was only detected in G1 in the 11% of the placentomes. However, at 28 dpi the frequency of detection in placentomes was higher in G1 (96%) than in G2 and G3 (7% and 47%, respectively) besides in fetuses was lower in G2 (20%) than in G1 and G3 (100% and 87%, respectively). Regarding late abortions, stillbirths, and lambs of G1, G2 and G3, the frequency of microscopic lesions was similar between groups (79%, 78% and 67%, respectively) whereas *T. gondii*-DNA was evidenced in 100%, 55% and 100%, respectively. These recently obtained *T. gondii* isolates led to similar reproductive losses but intra- and inter-genotype variations in the rise of hyperthermia, dynamics of antibodies, frequency of lesions and parasite detection and distribution

Conclusions

Genotype and phenotypic traits of each *T. gondii* isolate has an important role on the outcome of infection in ovine toxoplasmosis and mechanisms responsible for it need further investigations

A97

Toxoplasma gondii infection-driven DNA damage and chromosome instability in human host cells

Z. Velásquez[1], L. Rojas Barón[1], I. Conejeros[1], S. Butterworth[2], M. Treeck[2], C. Hermosilla[1], A. Taubert[1]

[1]Justus-Liebig-Universität Gießen, Institut für Parasitologie, Gießen, Germany
[2]The Francis Crick Institute, Signalling in Apicomplexan Parasites Laboratory, London, United Kingdom

Introduction: The obligate intracellular apicomplexan parasite *Toxoplasma gondii* is a globally spread zoonotic protozoa causing severe health problems in humans and animals. Prenatal infections may cause abortion or severely affect progeny welfare, and acute *T. gondii* infections may become life-threatening in immunocompromised patients. Recently, we reported that *T. gondii* infection induces an increased percentage of binucleated cells, host cell chromosome segregation defects and cytokinesis failure in bovine endothelial cells, which also showed cell cycle arrest in S-phase, suggesting that parasite infection may directly interfere with host DNA synthesis or impair DNA quality. Objectives: Our aim here was to study if *T. gondii* infection induces host cell DNA damage. Materials & methods: DNA was quantified via FACS-based approaches. DNA damage was studied via comet assays and immunofluorescence-based detection of typical markers for DNA damage foci (γH2AX), S- (PCNA) and G2-phase (Geminin). Protein expression of ataxia-telangiectasia mutated (ATM)- and ATM-and Rad3 (ATR)-related key markers was assessed by Western blotting. Intracellular and extracellular ROS was quantified via DCHF-DA- and AmplexRed assay. Results: *T. gondii* infection led to host cell S-phase arrest, binucleated cell phenotype and micronuclei formation already 15 min after the invasion. Both, comet assays and γH2AX detection revealed that *T. gondii* triggered DNA double-strand breaks shortly after infection and at a higher percentage in S-phase. However, ROS levels were not changed between 15-180 min p. i. The results showed that the DNA damage response (DDR)-, ATM-dependent pathway was activated in *T. gondii*-infected cells. Interestingly, effector molecules of the overall DDR pathway like phospho-p21 and cyclin E1 were also found enhanced after infection. To address the role of cyclin E in this process, a *T. gondii* mutant (RHΔhce1, RHΔmyr1), which fails to induce host cell cyclin E1 expression, was here used for infection. Overall, infections with mutated tachyzoites induced host cell DNA damage equal to control parasites, thus denying cyclin E-dependent reactions. Conclusion: Current data suggest that *T. gondii* triggers genome instability and DNA strand breaks early after infection. Both processes are neither dependent on host cellular ROS or cyclin E1 upregulation.

A98

Characterisation of a disordered nuclear effector protein that interacts with FOXO1 in *Theileria*-transformed cells

K. Woods[1], A. Naguleswaran[1], F. Brühlmann[1], M. Maurizio[1], M. W. Schmid[2], P. Capewell[3], W. Weir[3], T. Karagenç[4], B. Brian[3], P. Olias[1,5]

[1]University of Bern, Bern, Switzerland
[2]MWSchmid GmbH, Glarus, Switzerland
[3]University of Glasgow, Glasgow, United Kingdom
[4]Aydın Adnan Menderes University, Aydin, Turkey
[5]Justus-Liebig-Universität Gießen, Gießen, Germany

Theileria are tick-borne apicomplexan parasites that infect bovine leukocytes and cause Tropical Theileriosis. Within a few days of infection, parasitized leukocytes acquire a transformed phenotype comprising uncontrolled proliferation, resistance to apoptosis, immortality and increased invasiveness. This phenotype depends strictly on the presence of a viable parasite. RNA sequencing allowed us to identify extensive gene expression changes in primary macrophages following infection with *Theileria*. These include an upregulation of genes involved in DNA replication, cell cycle and translation, and a downregulation of genes involved in adhesion and innate immune response. To try to understand how *Theileria* modulates host gene expression networks, we used a comparative bioinformatics approach to identify *Theileria*-encoded effector proteins that are exported into the host nucleus. To facilitate this we performed Oxford Nanopore and Illumina whole genome sequencing on seven *T. annulata* clones isolated from different geographical regions. We analysed features such as selective pressure (dN/dS ratio) and protein disorderedness to identify and validate novel *Theileria* effector proteins. One of the identified proteins, TaC12_008960, is highly enriched in the host nucleus where it binds to the transcription factor Forkhead box protein O1 (FOXO1). FOXO transcription factors regulate many cellular processes including nutrient metabolism, DNA damage response, autophagy, cell cycle progression and oxidative stress response. FOXO1 expression is increased following infection, and immunofluorescence and western blot analysis confirmed that FOXO1 is localized in the nucleus of infected cells. We are exploring the functional significance of this interaction and testing what effect TaC12_008960 nuclear expression has on FOXO1 activity.

A99

Theileria annulata NIDP2 belongs to a novel secreted protein family and interacts with the tumour suppressor STAG2 and the p53-regulating protein NOC2L

F. Brühlmann[1], C. Perry[1], C. Griessen[1], A. Naguleswaran[1], K. Woods[1], P. Olias[1,2]

[1]University of Bern, Bern, Switzerland
[2]Justus-Liebig-Universität Gießen, Gießen, Germany

Theileria annulata is a tick-transmitted apicomplexan parasite that causes a fatal cancer-like disease in the bovine host. The transient malignant phenotype is not based on genome instability or mutations at the DNA level as in cancer cells. We hypothesized that exported parasite proteins are responsible for the observed alteration of host signaling pathways in infected leukocytes. Only few exported proteins have been identified so far. To reveal uncharacterized *Theileria* effector proteins we used the TurboID proximity labeling technology and targeted the host cell nucleus and parasite membrane. By this approach we identified a novel repetitive protein locus of four intrinsically disordered proteins uniquely present in transformative *T. annulata* and absent in the non-transformative species *T. orientalis*. Co-immunoprecipitation experiments revealed an interaction of the exported protein NIDP2 with the host proteins NOC2L and STAG2. NOC2L is a protein involved in tumor suppressor p53 silencing. The STAG2 gene, another known tumor suppressor, encodes a subunit of the cohesin complex, which is involved in sister chromatid cohesion, with important roles in chromatin structure maintenance, gene expression and DNA repair. Further investigations are now being undertaken to analyze the contribution of these interactions to host cell transformation. The latest results will be presented at the conference.

.

Session XVII – Vectors and Entomology 1

A100

Hibernation site patterns and survival rates of adult overwintering mosquitoes in Central Europe

F. G. Sauer[1], E. Timmermann[2], U. Lange[1], E. Kiel[2], J. Schmidt-Chanasit[1], L. Myczko[3], R. Lühken[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Arbovirology, Hamburg, Germany
[2]Carl-von-Ossietzky University, Aquatic Ecology and Nature Conservation, Oldenburg, Germany
[3]Poznań University of Life Sciences, Department of Zoology, Poznań, Poland

Adult overwintering mosquitoes can act as reservoir for viruses (e.g. West Nile virus) and thus may contribute to the establishment of mosquito-borne viruses in Central Europe. Knowledge on the ecology of adult overwintering mosquitoes can enhance our understanding of the mosquito population development after winter and how arboviruses persist in temperate regions. Here, we present three studies analysing mosquitoes" preferred hibernation sites and the factors influencing the winter survival in these hibernation sites. In the first study, we quantified the number of adult overwintering mosquitoes in cellars and aboveground structures such as sheds or barns. In 2016/17 and 2018/19, we searched for overwintering mosquitoes in 149 different constructions in Northwest Germany. Over both winters, 2,716 mosquitoes were collected; mosquitoes were detected in 44% of the cellars and 33% of the aboveground constructions. *Culex p. pipiens* Linnaeus, 1758 was the dominant species in cellars, while high numbers of *Anopheles messeae* Falleroni, 1926 were detected in a single barn. Only nine *Culex torrentium* Martini, 1925 specimens were detected, although collection data from summer would suggest that the species is highly abundant. In 2022, a second study was conducted in Poznań, Poland, where we collected overwintering mosquitoes from animal burrows and spatially close man-made constructions. In total, we detected 5,511 *Culex* specimens (man-made sites: 3,823, animal burrows: 1,688). While *Cx. p. pipiens* was again the most abundant species at the man-made hibernation sites (~90%), *Cx. torrentium* predominated in the animal burrows (~75%). The third study aimed to analyse the survival rates in dependence of the environmental conditions at different hibernation sites. Field-sampled overwintering *Cx. p. pipiens* and *An. messeae* were divided into groups and placed in cages at different cellars and sheds, where temperature and relative humidity was logged hourly. Mosquito wing size, lipid content and mortality rates were measured. Increasing mean temperatures at the hibernation sites (5 °C to 16 °C) but not mean relative humidity (58% to 94%) were correlated to the winter mortality rates of the mosquitoes. Larger specimens store more lipids, and in *Cx. p pipiens*, but not in *An. messeae*, survival probability of large specimens was significantly higher.

A101

Accuracy of the automated mosquito counting device BG-Counter 2

L. Rauhöft[1], F. G. Sauer[1], S. M. Martins Afonso[1], T. Șuleșco[1], J. Schmidt-Chanasit[1,2], R. Lühken[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Arbovirologie, Hamburg, Germany
[2]University Hamburg, Hamburg, Germany

The transmission of pathogens is an important capability of mosquitoes. Prevention methods such as the application of insecticides or the removal of potential breeding sites are common practice. However, the timing of their application requires exact knowledge on the spatial-temporal occurrence of the vector species. Mosquitoes are usually collected with CO_2-baited traps and subsequently identified by morphological and molecular biological methods. This procedure is very time consuming, which prevents a short-term assessment of the risk of local mosquito-borne pathogen transmission. Automatic counting traps allow a timely decision-making on the prevention measures against pathogen transmission. The BG-Counter 2 (Biogents) counts insects and discriminates these by size directly in the field. Placed on CO_2-baited traps, captured insects are categorized as "mosquito sized", "too small to be a mosquito" and "too large to be a mosquito". The Counter additionally measures standard environmental parameters, such as temperature and humidity. All information is transmitted to a cloud server at 15-minute intervals. In order to assess the accuracy of this counting device, traps were placed all over Germany which were run in daily and bimonthly intervals. The BG Counter 2 was attached to a CO_2-trap (BG-Trap-Station) and a modified version for gravid mosquitoes. All captured mosquitoes were identified morphologically. The number of mosquitoes were compared to the output of the counter. Spearman correlation coefficients between captured and counted mosquitoes and the counting accuracy was calculated. The correlation varied greatly depending on sampling site (r_s=0,33-0,72). During months with high mosquito abundances, the correlation was also the highest (r_s=0,66). The lowest correlation could be seen in early spring and autumn, during low mosquito abundance months (r_s=0,33). While the BG-Counter 2 can depict the general phenology well, the accuracy during low mosquito abundance is rather low.

A102

Host-feeding patterns of European mosquitoes in relation to land use

F. G. Sauer[1], T. Șulești[1], L. Jaworski[1,2], J. Börstler[1], A. Tomazatos[1], E. Kiel[2], N. Becker[3,4], D. Cadar[1], J. Schmidt-Chanasit[1,5], R. Lühken[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]Carl-von-Ossietzky University, Oldenburg, Germany
[3]Institute of Dipterology (IfD), Speyer, Germany
[4]Heidelberg University, Heidelberg, Germany
[5]Universität Hamburg, Hamburg, Germany

Introduction

Mosquitoes (Diptera: Culicidae) are the most important arthropod vectors of pathogens. Understanding their blood-feeding behavior is an essential element to assess the vectorial capacity of a mosquito population. Therefore, this study aims to analyze mosquito spatial-temporal host-feeding patterns by barcoding blood-fed female mosquitoes.

Methods

Between 2012 and 2019, mosquitoes were sampled at different sites in Germany, Iran, Moldavia and Romania. Blood-fed specimens were analyzed by PCR with vertebrate specific primers and subsequently processed with Sanger sequencing. The sequences were compared to sequences from GenBank to identify the host species. The host specificity of the mosquitoes was tested by calculating the checkerboard score (C-score). In addition, land use information based on the Copernicus global land cover layers were extracted from each sampling site to analyze its influence on the mosquito-host interaction.

Results

In total, we collected 4,827 blood-fed specimens of 48 different mosquito taxa. The molecular biological analysis revealed 82 host taxa, including 3 amphibia, 42 bird, 36 mammal and 1 reptile species. The five dominant host species were domestic cattle (34%), human (18%), pig (13%), horse (9%) and roe deer (6%). C-scores indicated a random structure in the host-feeding patterns, i.e. mosquitoes use a broad range of different host species. Nevertheless, most mosquito species preferred certain host groups. Species from the genus Aedes predominantly fed on mammals, while we detected a balanced proportion of mammal and bird blood in Culex specimens. The analyses showed that the mosquito-host interaction is significantly affected by land use factors, e.g. at forest sites, human blood was predominantly detected in Aedes mosquitoes, while at unforested sites, particularly in urban areas or in shrublands, humans are more likely to be bitten by Culex mosquitoes.

Conclusion

Land use changes can influence the abundance and species composition of mosquitoes and vertebrates affecting vector-host dynamics. This large data with a standardized molecular screening method gives a unique insight in the spatial-temporal host-feeding patterns of mosquitoes and the relevance for mosquito-host interaction on the epidemiology of mosquito-borne diseases.

A103

Developing a hybrid model for early-warning against mosquito-borne diseases in Germany

S. M. Martins Afonso[1], L. Rauhöft[1], M. L. Wehmeyer[1], T. Șulești[1], J. Schmidt-Chanasit[1,2], F. G. Sauer[1], R. Lühken[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Arbovirology, Hamburg, Germany
[2]Universität Hamburg, Faculty of Mathematics, Informatics and Natural Sciences, Hamburg, Germany

Driven by globalisation and climate change, mosquito-borne viruses have emerged in the last decades, with Usutu virus (USUV) transmission first detected in Germany in 2010, and West Nile virus (WNV) in 2018. Mechanistic (e.g., epidemiological R_0-models) and correlative (e.g., environmental niche models, ENMs) modelling approaches are commonly used to evaluate the risk of transmission, but there is a gap in literature that integrates both streams for more informative results. Most studies also employ static data instead of regularly updated data, limiting their ability to make near-real time predictions and guide mosquito control measures. In this study, a "hybrid" model was developed in which estimates of the spatial-temporal abundance of mosquito populations generated from ENMs were used to refine a mechanistic R_0 model based on temperature-dependent and taxa-specific transmission parameters. Real-time mosquito surveillance data collected from traps across Germany provided an accurate estimate of vector abundance, and allowed deriving the vector-to-host ratio parameter fed into the model. Nation-wide climate data updated on an hourly basis also served as model input to yield short-term forecasts presented as risk maps, which can be easily interpreted and used as a tool for risk assessment. Results so far suggest that the role of *Culex torrentium* in WNV transmission may have been grossly underestimated, as its high vector competence for this virus generated high R_0 values. Integrative models that take advantage of regularly updated climate and mosquito surveillance data could be of great value to better guide decision-making by public health authorities regarding surveillance plans and preventive measures.

A104

Distribution of *Dermatobia hominis* and *Cochliomyia hominivorax*: A Gateway to Myasis Prevention in Colombia

J. Donato-R[1], A. Donato-R[2], O. L. Pardo[3]

[1]Universidad Nacional de Colombia, Departamento de Biología, Bogotá, Colombia
[2]Unidad Médica Integral María Auxiliadora, Radiologist, Bogotá, Colombia
[3]Unidad Médica María Auxiliadora, Dermatologist, Bogotá, Colombia

Background: Myasis, a vector-transmitted disease, is a persistent public health problem in South America, where it is most commonly transmitted to humans by the fly larvae Dermatobia hominis (Dh) and Cochliomyia hominivorax (Coh) of the order Diptera. In Colombia, the geographic distribution of Dh and Coh is little known, and clinical reports of myasis are scarce. Results: Based on an exhaustive review of the biological-medical literature, biological collections, and clinical case records, the geographic distributions of Dh and Coh in Colombia were determined by province; the history of the myasis literature in Colombia was sketched; and the typical clinical presentation of myasis in Colombia was summarized. Conclusions: Myasis is endemic in Colombia and is concentrated in the cities and provinces of the country´s Andean Mountain ranges, where the majority of its population and primary medical care services are also found. Key Words Myasis, fly larvae, geographic distribution, Andes, Colombia.

A105

Ultrasound assessment for hepatosplenic schistosomiasis in a highly endemic area of Madagascar: assessing the needs to call for actions

V. Marchese[1,2], T. R. Rafaralahivoavy[3], S. H. Razafimahefa[4], P. Klein[1], T. Randrianarisoa[5], J. Richter[6], J. May[1,2], D. Fusco[1,2], R. A. Rakotoarivelo[5,7]

[1]Bernhard Nocht Institute for Tropical Medicine, Department of Infectious Diseases Epidemiology, Hamburg, Germany
[2]German Center for Infection Research (DZIF), Hamburg, Germany
[3]University Hospital of Andrainjato, Radiology Department, Fianarantsoa, Madagascar
[4]University of Fianarantsoa, Gastroenterology Department, University Hospital Andrainjato, Fianarantsoa, Madagascar
[5]University Hospital Tambohobe, Infectious diseases Department, Fianarantsoa, Madagascar
[6]Charité – Universitätsmedizin Berlin, Institute of Tropical Medicine and International Health, Berlin, Germany
[7]University of Fianarantsoa, Faculty of Medicine, Fianarantsoa, Madagascar

Introduction
Ultrasound (US) is crucial for the assessment of hepatosplenic schistosomiasis morbidity (HSS), although it is still poorly implemented in endemic countries, such as Madagascar. HSS is therefore mostly detected with complications (i.e., variceal bleeding, ascites) when praziquantel treatment is not effective, while some regression has been shown in lower degrees of periportal fibrosis.

Objectives
The overall objective is to describe US patterns and clinical presentation among patients with hepatosplenic schistosomiasis in a highly endemic area of Madagascar in order to advocate for the need of US implementation in Madagascar for both early detection and morbidity control of chronic schistosomiasis.

Materials & methods
In a descriptive design we aim at recruiting 60 patients among all adult patients attending primary health care, pre-screened using urine based rapid diagnostic test regardless symptom presentation. Recruitment is currently ongoing. Morbidity is assessed following WHO guidelines for image pattern (IP) classification: no fibrosis (A), borderline aspect/ incipient portal fibrosis (B) (both no fibrosis group), moderate peripheral fibrosis (C), moderate central fibrosis (D) (moderate fibrosis group), and severe fibrosis (Dc, E, Ec,-F). Bleeding risk (BR) is assessed with SMS score using IP classification and portal vein quotient. Those presenting an SMS score $\geq$ 2 undergo EGDS and additional lab tests for further management.
All patients are tested for chronic viral hepatitis, HIV antibodies, transaminases, blood count, albumin and creatinine. Praziquantel 40 mg/kg is administered once or for three days based on BR. US classification will be validated by two external experienced sonographers through imaging evaluations.

Results
Up to December 2022, eight patients (six women, age range 21-73 years, no viral infections) underwent US evaluation, with the following results: three severe forms (one Dc pattern, two F patterns, one of them with portal thrombosis), four moderate fibrosis (three C pattern, one D pattern) and one patient with no fibrosis. With the exception of one patient with severe fibrosis, none of them referred previous history of gastric bleeding, nor severe symptoms.

Conclusion
Our preliminary results show advanced fibrosis in patients without severe symptoms, supporting the need for early identification of HSS, for which US evaluation remains the first diagnostic tool.

A106

Prevalence of schistosomiasis and risk assessment among farmers in rural Madagascar: a cross-sectional study

A. Remkes[1], R. Krumkamp[1,2], T. Rasamoelina[3], R. Razafindrakoto[3], N. M. Nantenaina[3], J. Hainasoa[4], N. Jouanard[5], P. Martel[5], V. Marchese[1], N. Fischer[1], J. Saalfrank[1], J. May[1], R. Rakotozandrindrainy[6], R. A. Rakotoarivelo[4], D. Fusco[1,2]

[1]Bernhard Nocht Institute for Tropical Medicine, Department of Infectious Diseases Epidemiology, Hamburg, Germany
[2]German Center for Infection Research (DZIF), Hamburg, Germany
[3]Centre d'Infectiologie Charles Mérieux (CICM), Antananarivo, Madagascar
[4]University of Fianarantsoa, Department of Infectious Disease, Fianarantsoa, Madagascar
[5]APDRA Pisciculture, Massy, France
[6]University of Antananarivo, Department of Microbiology, Antananarivo, Madagascar

Introduction: Schistosomiasis is a parasitic disease of poverty spread by freshwater snails. Humans become infected through contact with contaminated freshwater. Therefore, occupational activities such as rice cultivation are believed to be a risk factor due to long and regular contact with contaminated water. It is to be noted that 70 percent of Madagascar's population is engaged in agriculture, mainly rice cultivation.

Objectives: The present study aims to investigate the prevalence of schistosomiasis and its risk factors in rural Madagascar.

Patients & Methods: A cross-sectional study was conducted in 44 villages in the Vatomandry District. 1204 households were randomly selected based on their accessibility and agricultural profile, with a maximum of 5 participants per household. A total of 5199 participants were included meeting following inclusion criteria: voluntary consent, age over 5 years, no fever or Covid vaccination in the last 2 weeks, and no pregnancy. Door-to-door surveying was used to obtain sociodemographic background and dietary behaviour data based on the "UN Guidelines for Measuring Household and Individual Dietary Diversity". 5196 urine and 995 blood samples were collected for Schistosome infection analysis by means of urine based rapid diagnostic tests (RDT) and PCR.

Results: A total of 1204 households with a median household size of 5 members (IQR=5-6) were surveyed. Among participants (n=5199), 56.1% were female. The mean age was 27 years (IQR=15-44), with 29.8% under 18. 58.1% of the total sample tested positive for schistosomiasis by RDT. 45.5% of adults work in agriculture. 58.5% of women and 57.6% of men tested positive. 45.4% of those under 18 tested positive, whereas 63.5% of adults did. Among adults who reported being farmers, 73.4% tested positive, 56.1% of non-farmers, and 43.5% of unemployed. 14.2% reported having been treated against schistosomiasis in the past, of whom 74.2% reported having been treated more than 12 months ago.

Conclusion: Our preliminary data show that a high proportion of the population tested positive by RDT for schistosomiasis. The highest proportion of individuals testing positive was among farmers, who were also by far the largest occupational group. The positive rate among men and women was almost identical. Additional analysis is underway to further identify the most common species and risk factors for infection. This study will help improve preventive measures to control schistosomiasis.

A107

Red fox as a reservoir of *Cryptosporidium* spp. and *Giardia intestinalis* in urban and natural areas

D. Dwużnik-Szarek[1], M. Jackowiak[2], E. Mierzejewska[1], A. Bajer[1]

[1]University of Warsaw, Department of Eco-Epidemiology of Parasitic Diseases, Warsaw, Poland
[2]Institute of Environmental Protection, Central Laboratory for Environmental Analysis- CentLab, Warsaw, Poland

Introduction: Red fox (*Vulpes vulpes*) is a major host for various intestinal parasites, however its role in spreading protists from the genera *Cryptosporidium* and *Giardia* is poorly understood, especially in urban environment.

Objectives: Main aim of presented study was to compare prevalence of *Cryptosporidium* spp. and *Giardia intestinalis* infection in foxes from natural habitats and Warsaw parks.

Materials & methods: fecal samples obtained from 88 foxes (44 obtained during the hunting season 2016-2018 and 44 from Warsaw parks) were screened for *Cryptosporidium* spp. and *G. intestinalis* presence. For the detection of both intestinal parasites, the commercial immunofluorescent assay MeriFluor *Cryptosporidium*/*Giardia* was used. For *Cryptosporidium* detection modified Ziehl-Neelsen (ZN) staining of faecal smears and PCR amplification and sequencing of *18S rDNA* and *60kDa* glycoprotein gene (*GP60*) were used. For *Giardia* detection, PCR amplification and sequencing of the glutamate dehydrogenase (*gdh*) and the *SSU-rRNA* genes were conducted.

Results: Our results showed differences between prevalence of *Giardia* and *Cryptosporidium* among foxes from natural and urban areas. There species of *Cryptosporidium* (*C. hominis*, *C. parvum*, *C. canis*) and *Cryptosporidium* sp. rodent genotype were identified in foxes. Prevalence of *G. intestinalis* was much lower than prevalence of *Cryptosporidium* spp.

Conclusion: The obtained results expand the knowledge on zoonotic reservoir of *Cryptosporidium* and *Giardia* in two environments, which might help to determine public health threat associated with these protists.

Acknowledgements: The study was financially supported by National Science Centre (NCN) Preludium grant no 2019/35/N/NZ7/01772 (DDS)

A108

Development and testing of new multi-locus sequence typing procedures for potentially zoonotic *Giardia duodenalis* genotypes

P. Gosten-Heinrich[1], E. Radam[1], K. Winter[1], S. Caccio[2], T. Aebischer[1], C. Klotz[1]

[1]Robert-Koch-Institute, Berlin, Germany
[2]Istituto Superiore di Sanità, Rome, Italy

Introduction

Giardia duodenalis represents a species complex of 8 genetically distinguishable assemblages (A-H) with distinct host preferences. The two assemblages A and B are relevant to humans and are also found in a broad range of various mammals. Adequate procedures for molecular typing are only insufficiently developed to address epidemiological questions. Typing schemes require improvement of new marker genes allowing higher typing resolution, and need to account for allelic sequence heterozygocity (ASH) that can be a consequence of the tetraploid nature of the parasite or possible mixed infections.

Objectives

The aim was to test or develop new multi-locus sequence typing (MLST) procedures for assemblage A and B, respectively, as separate approaches are necessary for both assemblages.

Materials & methods

For assemblage B, based on newly generated genomes we developed a new MLST scheme comprising 7 genomic marker sequences. Nested PCR protocols were designed and the new procedure was evaluated on > 80 assemblage B isolates from various origins. For assemblage A, a recently published MLST procedure based on 6 marker genes was evaluated using > 70 human and animal derived isolates.

Results

The new assemblage B MLST scheme confirmed ASH as a hallmark of assemblage B, which occurred in approximately 70% of the isolates and further revealed a subpopulation of assemblage B parasites exhibiting very low to no ASH. Sequence analysis showed that these subpopulations clustered separately. Strikingly, sequences of isolates from an assemblage B outbreak clearly formed a sub-cluster within the high ASH cluster with significantly lower inter se SNP variation compared to non-outbreak high ASH cluster isolates.

The MLST scheme for assemblage A confirmed largely the lack of ASH in this assemblage and showed that sub-assemblage AII comprises only isolates from humans. All isolates from animals and a few from humans belonged to sub-assemblage AI. The discriminatory power within sub-assemblage AII was sufficient to group isolates from two separate outbreaks as distinct MLSTs.

Conclusion

Different typing protocols for assemblage A and B are necessary to retrieve informative MLST data for epidemiological purposes. The evaluation of relatedness of isolates based on SNPs depends on their respective subpopulation assignments. Both protocols will help to foster the generation of meaningful typing data much needed for future epidemiological studies.

A109

Schistosoma infection and risk factors among schoolchildren in the rural area of the Democratic Republic of the Congo

S. Linsuke[1], I. Gillon[2], M. Diasonama[3], J. D. Nzita[3], P. Mbala[2], P. Lutumba[4], J.-P. Van Geertruyden[5]

[1]National Institute of Biomedical Research (INRB), Epiodemiology, Kinshasa, Congo, The Democratic Republic Of The
[2]National Institute of Biomedical Research (INRB), Parasitology, Kinshasa, Congo, The Democratic Republic Of The
[3]Health Zone of Kwilu-Ngongo, , Kongo-Central, Congo, The Democratic Republic Of The
[4]University of Kinshasa, Tropical Medicine, Kinshasa, Congo, The Democratic Republic Of The
[5]University of Antwerp, Global Health Institute, Faculty of Medicine, Antwerp, Belgium

Introduction: Schistosomiasis (SCH) remains a major public health problem in the Democratic Republic of the Congo (DRC), especially in the rural community where environmental conditions increase the risk. The primary SCH control intervention strategy in the country is preventive chemotherapy with Praziquantel in all schoolchildren. This strategy was implemented in some endemic areas of the DRC but in other areas, there is a need of more scientific evidence on the burden of SCH for decision-making to optimize SCH control. The study aims to document the Schistosoma infection among schoolchildren in rural Health Area.

Methodology: A cross-sectional study was carried out from June to August 2021 on schoolchildren in the rural community of Kisangi in the South-West of the DRC. The helminth infection was assessed using duplicate Kato-Katz thick smears for stool and a standard filtration technique for urine. A finger prick blood sample was collected and to assess for malaria infection and hemoglobin (Hb) level. Socio-demographic data and risk factors were assess using a semi-structured questionnaire.

Results: Out of 480 schoolchildren aged 5 to 14 years enrolled in the study, 268 (55.8%; 51.4-60.2) had Schistosoma infection. Infection with S. haematobium, S. mansoni, and both infections were detected in 197 (41%; 36.6-45.5), 103 (36.3%; 31.9-40.6), and 103 (38.4%; 32.5-44.3) schoolchildren, respectively. The overall prevalence of malaria infection, anemia, and malnutrition were 16.9% (13.5-20.2), 49.4% (44.9-53.9), and 37.5% (33.2-41.8), respectively. Mean Hb level of all participants was 11.6 g/dl ±1.5. On multivariate regression analysis, Schistosoma infection was significantly associated with age between 9-14 years (aOR. 2.3; p<0.001), and anemia (aOR. 3.4; p<0.001). However, malaria infection (aOR. 0.5; p=0.016) was a protective Schistosoma infection factor.

Conclusion: Our findings indicate the hyper-endemic status of schistosomiasis associated with anemia among schoolchildren. There is a need for specific control intervention including prevention chemotherapy and supplementation in micronutrients to avoid anemia among schoolchildren.

Workshop IV – PoP

A110
The Physics of Parasitism

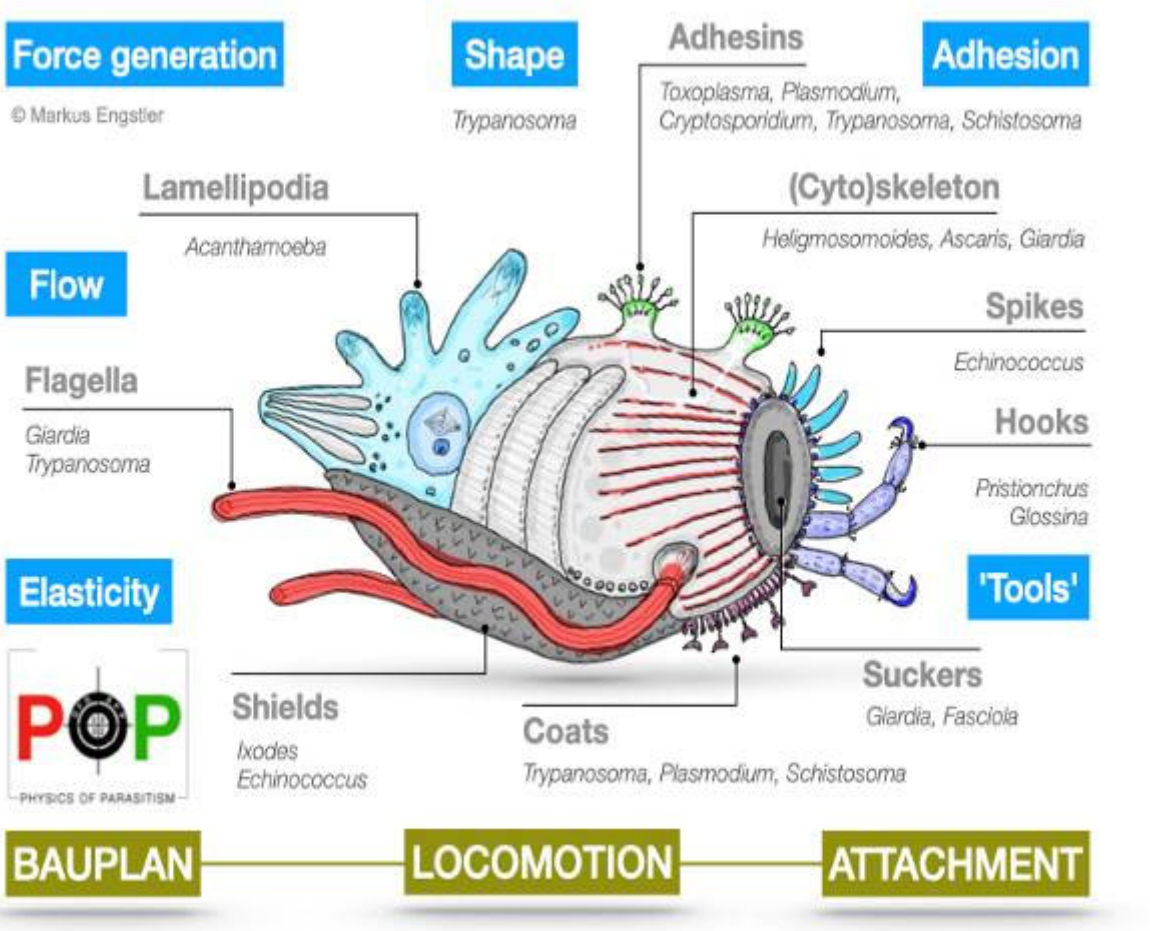
M. Engstler[1]

[1]Julius-Maximilians-Universität Würzburg, Zell- und Entwicklungsbiologie , Würzburg, Germany

Parasitism, the intimate antagonistic liaison between two species, has always fascinated scientists from a variety of disciplines. Traditionally, parasitology was concerned mainly with organismic studies, while today"s parasitology focuses on medically-relevant cellular and molecular mechanisms, at ever-increasing depth. The DFG priority programme "Physics of Parasitism" (PoP) defines a new frontier in this field, namely the physics of parasites interacting with their hosts. Parasitism has evolved many times and hence, there are numerous convergent solutions to the challenge of how to physically hijack a host. These long periods of co-evolution have equipped parasites with high degrees of optimality. Examples are parasitic tools such as suckers and shields, or refined locomotive devices that allow navigation and also attachment in various body fluids, in crowded and confined spaces, and in highly viscous environments - often at surprisingly high speeds. "Physics of Parasitism" opens new chapters in both parasitology and the physics of life. The results obtained during the interdisciplinary endeavour will expose novel ways of combating parasitic diseases based on mechanobiology, against which resistances are unlikely to evolve.

PoP focuses on three major physical/mechanical aspects of parasitism: (i) the construction and mechanical properties of the parasite bodies ("Bauplan"), (ii) the physics of parasite locomotion ("Locomotion"), and (iii) the physics behind mechanisms of host attachment ("Attachment"). In my lecture I will introduce and illustrate the concept of the "Physics of Parasitism" with examples.

Fig. 1


Force generation
© Markus Engstler
Shape
Trypanosoma
Adhesins
Toxoplasma, Plasmodium,
Cryptosporidium, Trypanosoma, Schistosoma
Adhesion
Lamellipodia
Acanthamoeba
(Cyto)skeleton
Heligmosomoides, Ascaris, Giardia
Flow
Spikes
Echinococcus
Flagella
Giardia
Trypanosoma
Hooks
Pristionchus
Glossina
Elasticity
'Tools'
PHYSICS OF PARASITISM
Shields
Ixodes
Echinococcus
Coats
Trypanosoma, Plasmodium, Schistosoma
Suckers
Giardia, Fasciola
BAUPLAN
LOCOMOTION
ATTACHMENT

A111

Atomic force microscopy-based characterization of *Giardia duodenalis* attachment

G. Gunaratnam[1], S. Reusch[2], B. Wieland[1], J. Dudek[3], M. Hannig[3], M. Bischoff[1], S. L. Becker[1], S. Dawson[4], C. Klotz[2], K. Jacobs[5,6], A. Aebischer[2], P. Jung[1]

[1]Saarland University, Institute of Medical Microbiology and Hygiene, Homburg, Germany
[2]Robert-Koch-Institute, Department of Infectious Diseases, Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany
[3]Saarland University, Clinic of Operative Dentistry and Periodontology, Homburg, Germany
[4]University of California Davis, Department of Microbiology and Molecular Genetics, Davis, CA, United States
[5]Saarland University, Experimental Physics, Saarbrücken, Germany
[6]Max Planck School Matter to Life, Heidelberg, Germany

Introduction

Trophozoites of the parasitic protozoan *Giardia duodenalis* (GD) have a strong attaching behaviour on artificial surfaces and on the epithelial surface of the human duodenum, a well-known prerequisite for GD infections. Despite the profound knowledge about the cellular structures involved in attachment and the pivotal roles of the ventral disc, the lamellipodium-like ventrolateral flange and the beating flagellas, the quantitative adhesive forces of single trophozoites have not been reported yet.

Objectives

Here, the maximum detachment forces (MDF) of single GD trophozoites on a smooth glass surface (RMS = 1.1 ± 0.2 nm) were determined. A direct comparison with the detachment behaviours of other eukaryotic species (i.e. *Candida albicans* yeast cells and human oral keratinocytes) was done to lay the foundation for a theoretical explanation of the attachment mode of GD.

Materials & Methods

Fluidic force microscopy (FluidFM)-based single-cell force spectroscopy was used to quantify the adhesion parameters of single GD WB C6 trophozoites to glass. Briefly, surface-bound trophozoites were loaded with a FluidFM micropipette, simultaneously sucked by negative pressure and gradually pulled and removed from the surface by retraction of the micropipette. Recorded force-distance curves were evaluated for the MDF, the cell-detachment length and curve characteristics. For comparison, experiments with *C. albicans* ATCC 10231 and K2 keratinocytes were conducted.

Results

MDFs for GD trophozoites were with 8.2 ± 4.3 nN comparable to the values determined for *C. albicans* (7.5 ± 4.6 nN), but clearly lower than those seen with oral keratinocytes (55.8 ± 43.9 nN). Interestingly, our GD retraction curves displayed a gradual force increase on the pulled cell until the MDF was reached close to the cell-detachment length. For the other investigated species, however, a clear distance between the MDF and the cell-detachment length was observed with intermediate detachment forces probably caused by the rupture of single or few binding partners. Importantly, this pattern was never detected for GD trophozoites.

Conclusion

Our data indicate a unique adhesion mechanism for GD trophozoites on artificial surfaces that likely are dependent on the ventral disc contributing a major part to the overall adhesion force. Research with GD ventral disc mutants and with porous or nanorough surfaces is ongoing to confirm this hypothesis.

A112

Investigation of Host-Parasite Interaction in *Giardia muris* infection via a fluorescent Ca^{2+} reporter

J. M. Liebeskind[1], L. Asmussen[2], S. Hartmann[3], A. E. Hauser[2,4], S. Rausch[3]

[1]Freie Universität Berlin, Berlin, Germany
[2]Charité – Universitätsmedizin Berlin, Berlin, Germany
[3]Freie Universität Berlin, Berlin, Germany
[4]German Rheumatism Research Center, Berlin, Germany

Introduction

Infections with the protozoan parasite *Giardia duodenalis* cause a global health burden with over 200 million symptomatic cases every year. *Giardia muris*, a natural parasite in mice, serves as a model for the asymptomatic course seen in many cases of *G. duodenalis* infection. Our previous work suggests that modest adaptive immune responses operate together with the intestinal accumulation of pro-inflammatory myeloid cells, allowing for the control of infection in absence of overt immunopathology. Whether attachment of *Giardia* trophozoites directly drives immune defense by cells forming the intestinal epithelial barrier is not clear.

Objectives

As macrophages develop a pro-inflammatory phenotype upon activation of the mechano-sensitive Ca^{2+} channel PIEZO-1, we address whether attachment of *G. muris* to myeloid cells results in mechano-sensing driven defense mechanisms at the site of infection.

Materials & Methods

Using 2-Photon microscopy, Bone Marrow Derived Macrophages (BMDM) from LysM-GCaMP6f reporter mice allow the visualization of PIEZO-1 induced Ca^{2+} influx based on fluorescence intensity. The performance of the system was tested by exposure of BMDM to Ca^{2+} ionophores, PIEZO-1 agonists and by direct mechanical stimulation applied by nanoindentation via Atomic Force Microscopy (AFM). Finally, BMDM were exposed to live *G. muris* trophozoites.

Results

Stimulation of BMDM with ionomycin caused an immediate increase in intracellular Ca^{2+}, which was blocked by the addition of Ca^{2+} chelators. The application of the PIEZO-1 specific agonist YODA caused a similar effect, which could be reverted by the application of YODA antagonist GsMTx4. Furthermore, mechanostimulation of BMDM by nanoindentation via Atomic Force Microscopy initiated an increase in Ca^{2+} signal in the stimulated cells. Importantly, this finding was mirrored by a clear increase in Ca^{2+} reporter signal intensity in BMDM exposed to live *G. muris* trophozoites.

Conclusions

The LysM-GCaMP6f system reports the activation of mechanosensitive Ca^{2+} channels in myeoloid cells. Whether the attachment process of *Giardia* trophozoites results in the PIEZO-1 dependent activation of myeoloid cells and thereby triggers a local defense program against *G. muris* infection remains to be investigated.

A113

Did protein engineering fail? Try biophysics! First structural insights into MEG-family proteins

S. Nedvedova[1], F. Guilliere[2], O. Walker[2], K. Peterková[3], J. Dvořák[4], A. E. Miele[2], M. Hologne[2]

[1]Czech University of Life Sciences, Department of Chemistry, Prague, Czech Republic
[2]Institut des Sciences Analytiques, BIOSYS, Villeurbanne, France
[3]Charles University Prague, Department of Parasitology, Prague, Czech Republic
[4]Czech University of Life Sciences, Center of Infectious Animal Diseases, Prague, Czech Republic

Schistosomiasis is a parasitic disease caused by trematodes affecting the lives of millions of people worldwide[1]. Our work focuses on *Schistosoma mansoni*, the most widespread species of schistosomes infecting humans. Adult parasites reside in the circulatory system and lay eggs that need transiting from the endothelium of the blood vessel via host tissues into the gut lumen to be excreted to perpetuate the life cycle[2]. However, many of them remain trapped in host tissues, where they induce inflammation and chronic debilitating pathology. Eggs produce many bioactive molecules[2,3], whose specific role is still yet to be characterized.

We decided to focus on a significantly upregulated group of proteins named MEG proteins (micro-exon gene). These proteins are unique not only because of their genetic structure but also because they are specific to Schistosomatidae[3]. Due to alternative splicing, almost all members of the more than 30 families of MEGs generate several isoforms[4]. They do not show relevant homologies to other proteins in the UniProt database[5], and their structure is still unresolved, as well as their specific role in egg-host interaction.

We selected members of the MEG-2 and MEG-3 families. Our approach using standard protein engineering tools to produce recombinant proteins failed to obtain enough material. Therefore, we decided to divide the sequences into short synthetic overlapping peptides. In particular, we subdivided 3 isoforms of the MEG-2.1 family into 8 peptides. We collected a series of 2D NMR homonuclear and heteronuclear spectra, measured in the natural abundance of 13C and 15N. The attribution of the peaks in the 2D spectra, in combination with distance geometry and energy minimization software, allowed us to determine the structures of these peptides belonging to 2 out of the 3 isoforms. Here we present the first structural information on *S. mansoni* MEG proteins, which can serve to infer the structure of other MEG family members. Resolving the structure is the first step toward the elucidation of MEGs role in host-parasite interactions.

1. WHO. Schistosomiasis. 2022 https://www.who.int/news-room/fact-sheets/detail/schistosomiasis

2. Costain AH et al. *Front immunol* 2018, 9: 3042.

3. Li XH et al. *PLoS NTD* 2018; 12.2: e0006235.

4. DeMarco R, et al. *Genome Res*. 2010; 20(8):1112-21.

5. UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res.* 2021; 49.D1: D480-D489.

A114

Visualizing the surface dynamics on and in *Trypanosoma brucei*

T. Mueller[1], E. Meiser[1], M. Engstler[1]

[1]Universität Würzburg, Zell- und Entwicklungsbiologie , Würzburg, Germany

Due to its extracellular lifestyle, *Trypanosoma brucei* is always exposed to the immune system of its host. While being able to switch their predominant variant surface antigen (VSG), their immune evasion strategy also heavily relies on the mobility of their surface coat, which enables them to remove antibodies from their cell surface within seconds via endocytosis. As the fast turnover on the surface must be mirrored in speed for degradation or recycling, we now also turn our attention to the inside of the cell.

We want to visualize the dynamics inside the endosomal network in three dimensions to elucidate how the sorting of membrane proteins is realized in a system, that does not have the time for expensive membrane budding and fusion process.

To measure the intracellular dynamics, we express an insect-stage specific membrane protein (EP1) in the bloodstream form of the parasite, which will stay confined to the endosomal compartments and the flagellar pocket. To selectively mark the protein, we Halo-tagged it. Living cells are labelled using picomoles of the dye, immobilized in hydro gel and imaged at 37°C. With the help of an astigmatic lens, we collect 3D information of our single emitters. The acquired data is processed using SMAP by Jonas Ries and reveals a resolution of 30 nm in x/y and 80 nm in z imaging at 25 Hz. The calculated positions of single emitters are then used to generate tracks using Swift by Ulrike Endersfelder. Those tracks are then analyzed using our custom software based on the SPTAnalysis by Pierre Parutto.

Preliminary data of Atto643-NHS labelled VSG shows that the flagellar membrane can act as a diffusional barrier that can not easily be crossed by surface molecules and that an accumulation of signal could be localized inside the cell in proximity to the flagellar pocket. Overall, the surface dynamics of the VSG of the 3D data seem to be comparable with previously acquired 2D data.

As the comparison of 2D and 3D data lead to a similar diffusion coefficient, the overall workflow for 3D data seems to be suitable. A challenge will arise with the spatially quite confined endosomal system to generate sufficient data for analysis, which will be compensated for by the long-lived Janelia Fluor dyes. In addition, our workflow would allow statistical analysis of the 3D track data, which could change the general understanding of the endocytotic flow and shed light on how the sorting and recycling mechanism really work.

A115

Optimal alignment of *plasmodium* merozoites at erythrocytes

A. K. Dasanna[1,2], S. Hillringhaus[2], G. Gompper[2], D. Fedosov[2]

[1]INM – Leibniz Institute of New Materials , Saarbrücken, Germany
[2]Forschungszentrum Juelich, Institute of Biological Information Processing-5, Juelich, Germany

Invasion of plasmodium merozoites into erythrocytes is an essential step for their survival and multiplication. The parasites align their apex with the membrane surface for successful invasion. It has been hypothesized that they use erythrocyte membrane deformability to efficiently align themselves. We use computer simulations to test the hypothesis. We model the merozoite as a hard egg-shaped particle and the erythrocytes using a continuum membrane model [1, 2]. Typical snapshot from computer simulations is shown in Figure 1. In this talk, I will discuss the role of erythrocyte deformability and parasite-membrane adhesion in the alignment process using multiscale modeling. We show that parasite-membrane interactions with an inhomogeneous contact interaction that describes an adhesion-strength gradient on the parasite plasma membrane lead to much faster alignment compared with experimentally measured timescales; pure rotational diffusion of the parasite leads to too slow alignment [3]. Therefore, we simulate the adhesion without a gradient but considering receptor-ligand bond dynamics [4]. I will show how the shape fluctuations and deformability of the erythrocytes along with the stochastic nature of receptor-ligand bond formation predict alignment times that are consistent with experimental observations. I will also discuss the role of parasite shape in the alignment process and show that original shape of the parasite performs better compared other shapes at various conditions [5]. Finally, I will provide an update on and outlook for our project on Toxoplasma gondii invasion into host cells within the SPP 2332 "Physics of Parasitism".

References:

[1] Fedosov, Dmitry, et al. "A multiscale red blood cell model with accurate mechanics, rheology, and dynamics." Biophysical journal 98.10 (2010): 2215-2225.

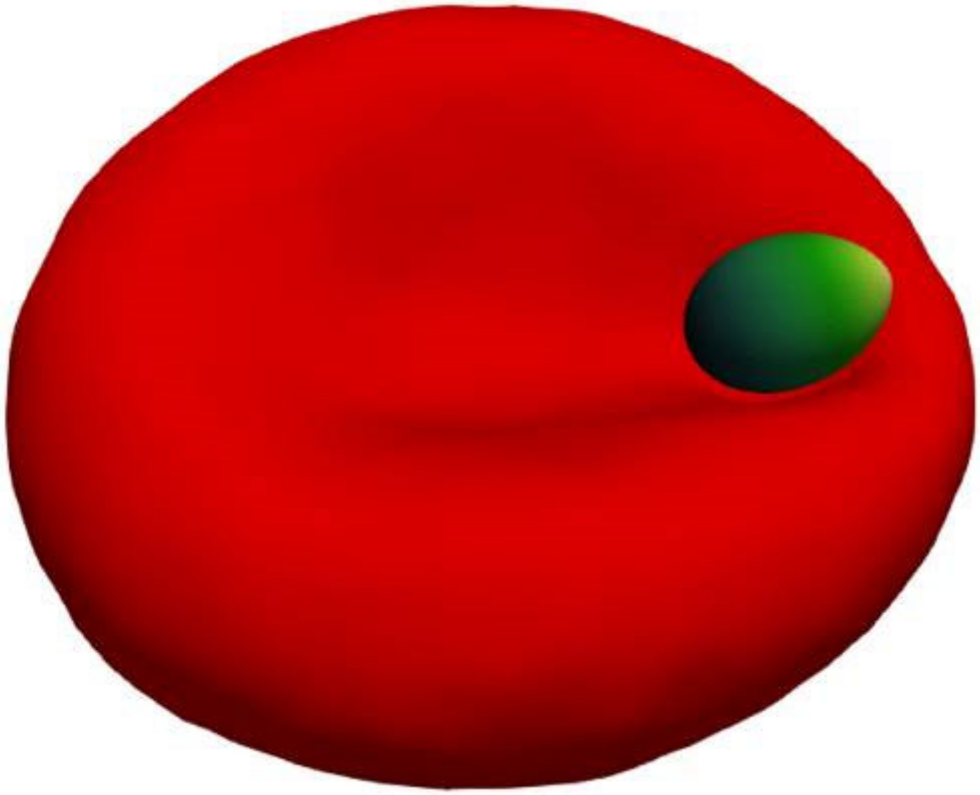
[2] Dasgupta, Sabyasachi, et al. "Membrane-wrapping contributions to malaria parasite invasion of the human erythrocyte." Biophysical journal 107.1 (2014): 43-54.

[3] Hillringhaus, Sebastian, et al. "Importance of erythrocyte deformability for the alignment of malaria parasite upon invasion." Biophysical journal 117.7 (2019): 1202-1214.

[4] Hillringhaus, Sebastian, et al. "Stochastic bond dynamics facilitates alignment of malaria parasite at erythrocyte membrane upon invasion." Elife 9 (2020): e56500.

[5] Dasanna, Anil K., et al. "Effect of malaria parasite shape on its alignment at erythrocyte membrane." Elife 10 (2021): e68818.

Fig. 1



A116

Biophysics of *Plasmodium* sporozoite migration

F. Frischknecht[1], M. Singer[1], K. Walz[1], A. Binder[1], J. Ripp[1], K. Quadt[1], L. Lettermann[1], U. Schwarz[1]

[1]Heidelberg University, Parasitology, Heidelberg, Germany

Plasmodium sporozoites are the forms of the malaria parasites transmitted by mosquitoes. They are slender, slightly curved cells that can migrate at high speed. While a number of proteins are known to be essential for migration, we have only a very limited understanding of the interplay between these. In order to migrate sporozoites need to attach to a substrate, generate force onto the substrate and to move forward and they have to detach the adhesive sites from the substrate. We have used a number of imaging and biophysics assays along with the generation of mutant parasites and will present some of these, such a laser trap and traction force experiments to measure force as well as 3D electron tomography to investigate the cytoskeleton of the parasite. Currently we are investigating the role of chiral elements in the migration of the parasite using a mix of in silico modelling, expansion microscopy and analysis of mutant parasites. These mutants lack actin or microtubule binding proteins that lead to non-chiral motility of the sporozoites.

A117

An infectious duo: The slender – stumpy success story

J. Lisack[1], C. Praisler[1], A. S. Kreis[1], M. Engstler[1]

[1]University of Würzburg, Department of Cell and Developmental Biology, Würzburg, Germany

The unicellular protozoan *Trypanosoma brucei brucei*, together with *T. vivax* and *T. congolense*, causes Animal African Trypanosomiasis (AAT) in livestock and wildlife in sub-Saharan Africa. These diseases are accompanied by a high degree of economic damage, affecting the low-income population severely. Since the parasite has a digenetic life cycle, the mammalian host is not the only organism it infects. Much of the *T. brucei* life cycle takes place in the arthropod vector, the tsetse fly (*Glossina spp*.). There are two forms of *T. brucei* in the circulation of an infected animal: the slender and stumpy bloodstream forms (bsf). The slender form proliferates, while the stumpy form is cell cycle-arrested. It is common knowledge that the stumpy forms rapidly differentiate to the first insect stage once being ingested by the tsetse fly. For more than a century it was assumed that the slender bsf dies in the tsetse midgut. It was only in 2021 that we showed that the slender bsf are perfectly capable of infecting the tsetse fly and going through the entire life cycle. Since quantitative experiments with vector infections are extremely complex, the question remained whether stumpy and slender forms are actually equally effective in colonising the tsetse fly. Therefore, we performed co-infection experiments with two differently fluorescent cell lines of *T. brucei*, one of which as slender form, the other as stumpy. These experiments were accompanied by *in vitro* analyses of the developmental transition of slender and stumpy trypanosomes to the insect stage using RNA-Seq. Furthermore, we tested if age of the flies makes a difference. It is thought that tsetse flies are less likely to get infected the older they become. Hence, infections of either slender or stumpy bsf were done with flies after their second or third feed. Altogether, we show that slender and stumpy bsf are equally successful in infecting the vector and going through the entire life cycle. In this process, slender forms do not become stumpy, but turn on specific genes to directly become the first insect stage (procyclic) in the midgut of the fly. Preliminary results also indicate that slender bsf, along with stumpy bsf, can also infect non-teneral flies. It can now be said without a doubt that the slender bsf of *T. brucei* are also able to infect the tsetse fly vector.

A118

The 3D architecture of the *Trypanosoma brucei* endosomes reveals the structural basis for rapid plasma membrane recycling

F. Link[1], A. Borges[1], M. Engstler[1]

[1]Universität Würzburg, Zell- und Entwicklungsbiologie , Würzburg, Germany

Trypanosoma brucei is an extracellular human and livestock pathogen that follows an effective strategy to evade the host immune system. This strategy relies on stochastical changes in the expression of variant surface glycoproteins (VSGs) that cover the entire cell surface of the parasite. Remarkably, trypanosomes recycle one cell surface equivalent within just 12 min and internalize surface bound antibodies within 2 min. Consequently, this parasite requires to have a highly effective endosomal sorting system. Currently, the endosomal system in *T. brucei* is described as distinct compartments that represent early, late and recycling endosomes. However, this model cannot explain the rapid rate of uptake and recycling in these organisms. Therefore, we analyzed the 3D architecture of the endosomal system.

For this purpose, we used different electron tomography and immuno-electron microscopic methods, as well as immunofluorescence and structured illumination microscopy (SIM) to analyse the 3D anatomy of the endocytic apparatus in *T. brucei*.

Electron tomograms show that the trypanosome endosomal system is more elaborate than previously thought and differs significantly from the mammalian endosome. Instead of being divided in distinct compartments it seems to consist of few continuous membrane systems. Antibodies against endosomal marker proteins (Rab5 – early endosomes, Rab7 – late endosome, Rab11 – recycling endosome) were used to perform in depth colocalization studies in wide-field microscopy. These analyzes indicate a high level of correlation between the different Rab proteins. To overcome the resolution limit of wide-field microscopy, super resolution microscopy and immuno-electron microscopy was used. All methods provide evidence for the existence of distinct functional endosomal subcompartments on the same membrane system, which questions the textbook picture of separate early, late and recycling endosomes.

Our results suggest that plasma membrane recycling in trypanosomes happens through a quick journey within an "endosomal highway", implying that its speed is diffusion-limited and not by trafficking through physically separated endosomal structures. Thus, we propose that the stream-lined architecture of the trypanosome endosomes is a consequence of the extreme and essential kinetics of plasma membrane recycling in these parasites.

A119

A quantitative mass spectrometry approach to identify novel proteins involved in macrophage infections by *Leishmania* parasites

N. Hagedorn[1], A. Fradera Sola[2], M. Mitnacht[1], T. Gold[3], E. T. Rauh[1], U. Schleicher[3], F. Butter[2], C. J. Janzen[1]

[1]University of Würzburg, Cell and Developmental Biology , Würzburg, Germany
[2]Institute of Molecular Biology, Quantitative Proteomics, Mainz, Germany
[3]University Hospital Erlangen, Microbiology-Institute Clinical Microbiology, Erlangen, Germany

Leishmania are single-cell parasites with an intracellular life cycle stage in phagocytic cells of their vertebrate hosts. Transcriptomic analyses of host cell defense mechanisms and how the parasites respond to these processes are already available. Due to the prevalence of post-transcriptional regulation in kinetoplastids however, transcriptomes do not reflect the protein composition very well.

Here we present a systematic comparative *in vitro* study of the infection process using a quantitative proteomics approach to uncover novel virulence factors.

Murine bone marrow-derived macrophages (BMDMs) were infected with 3 different species: *L. mexicana*, *L. major*, *L. infantum*. Whole cell protein extracts of parasites and BMDMs were prepared at 7 timepoints post-infection (0.5-72h) and were analysed using quantitative mass spectrometry. Candidate proteins were knocked out in *L. mexicana* and infection experiments were carried out to discover novel mechanisms involved in parasite adaptation during macrophage infections. Evaluation of infection experiments were carried out using fluorescence microscopy. An ImageJ macro was scripted to process images and automatically determine the number of infected macrophages.

We quantified the expression of approximately 2000 murine proteins in all 3 infection experiments, while 1500, 1000 and 1400 proteins were quantified for infections with *L. mexicana*, *L. major* and *L. infantum*, respectively. The number of differentially-expressed proteins during the time course of infection varied for each species (349, 133, and 287 proteins for *L. mexicana*, *L. major* and *L. infantum*, respectively). Our experiments identified many proteins putatively involved in the infection process, which were not found in previously published transcriptome analyses. For example, gene ontology enrichment showed 41 *L. mexicana* protein IDs involved in oxidoreductase activity. Additionally, we found 24 differentially-expressed proteins without any functional or structural annotation in the TriTryp database. Automated evaluation of infection experiments with two knockout cell lines showed reduced infectivity.

Our mass spectrometry approach identified novel virulence factors in *Leishmania* infections, that were not detected in transcriptomic screens. Automated evaluation of infection experiments successfully showed strong phenotypes for two knockout cell lines. Candidate proteins are being further investigated to decipher their role during the infections processes.

A120

Role of the CFTR in pathogenesis of *Giardia duodenalis* infections

A. Müller[1], T. Aebischer[1], C. Klotz[1]

[1]Robert-Koch-Institute, Department of Infectious Diseases, Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany

Introduction

As a widespread protozoal parasite, *Giardia duodenalis* is a common cause for abdominal pain, malabsorption and diarrhea. Intestinal epithelial barrier defects are frequently observed in patients affected by the multifactorial disease giardiasis, which is characterized by "leaky" barriers due to the disturbance of the tight junction complex. While the exact mechanisms remain unknown, recent findings suggest a novel chain of events amounting in epithelial barrier breakdown, which starts with a disturbed ion homeostasis during the early infection phase. Amongst these intestinal ion transporters, the anion channel CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) has been shown to be severly affected upon infection regarding its gene expression as well as its function.

Objectives

We aim to shed light on the putative role of the CFTR channel in *G. duodenalis* infections by investigating its impact on pathogenesis at different infection stages and processes such as the early altered ion homeostasis, disruption of the tight junctional complex and cellular differentiation (goblet cell hyperplasia) during the late infection phase.

Materials & Methods

Using the CRISPR/Cas9-technology, healthy intestinal organoids were converted into functionally impaired CFTR-mutants through insertion of the most common mutation (F508del) found in cystic fibrosis (CF) patients, a disease caused by mutations in the CFTR gene. For comparison of CFTR-impaired vs CFTR-functional organoids, mutant and wildtype were cultured in a compartmentalized transwell system and monitored regarding epithelial integrity throughout the infection period via transepithelial electrical resistance (TEER) measurements.

Results

Data from prior experiments show a robust transcriptional and functional downregulation of the CFTR ion transporter. Preliminary infection experiments with healthy and CF-patient derived intestinal organoids hint towards an increased prevalence of Clca1-positive (goblet) cells during the late infection phase in CF-derived cultures as compared to wildtype organoids.

Conclusion

Due to the significant downregulation of the CFTR channel during *G. duodenalis* infections and its pathogenic role in a variety of different diseases, we hypothesized that non-functionality of this important ion channel may benefit the parasite during infection. Experiments using CFTR-impaired organoids are still ongoing and will be discussed during the conference.

A121

Biological and immunological characterization of *Entamoeba histolytica*-derived extracellular vesicles

B. Honecker[1], V. Bärreiter[1], N. G. Metwally[1], K. Höhn[1], K. Harant[2], D. Cadar[1], B. Horváth[1], M. Er-Lukowiak[1], H. Lotter[1], I. Bruchhaus[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]Charles University Prague, Faculty of Science, Prague, Czech Republic

The protozoan parasite *Entamoeba histolytica* is the causative agent of amebiasis. While 90 % of intestinal infections remain asymptomatic, 10 % result in invasive disease. In some cases, parasites can invade the mucosa and migrate to the liver via the bloodstream, where amebic liver abscesses (ALAs) are formed. ALAs are deadly to humans if left untreated. Interestingly, ALA formation occurs more frequently in men compared with women. Onset of invasive disease is the result of parasite pathogenicity factors on the one hand and an overshooting immune response involving infiltrating monocytes and neutrophils on the other. To gain a better understanding of the interaction between parasite and host immune system, we investigate extracellular vesicles (EVs) as putative modulators of the immune response.

EVs were isolated from the supernatant of *E. histolytica* cultures using an ultracentrifugation-based approach. Biological characterization of the EVs was performed by proteomics, miRNA sequencing, nanoparticle tracking analysis and transmission electron microscopy. Two clonal cell lines differing in their pathogenicity were used for EV isolation. Analysis of the EV cargo revealed considerable differences between the two cell lines that might be relevant in the context of pathogenicity.

Analysis of putative immunostimulatory properties of these EVs was performed *in vitro* on primary murine monocytes and neutrophils from both male and female mice. RNASeq, flow cytometry and cytokine profiling were implemented to characterize the immune cell response. Stimulation of primary immune cells with EVs of both cell lines resulted in increased expression of activation markers on the cell surface and an increased secretion of pro-inflammatory cytokines, both in male- and female-derived cells. Regulation of genes involved in immune response was also observed using RNASeq. Taken together, these data suggest an involvement of EVs in the host immune response to *E. histolytica* infection.

A122

Applying high-resolution mass spectrometry imaging to visualize parasite-host interaction

K. R. Wiedemann[1], S. Gerbig[1], P. Ghezellou[1], M. Roderfeld[2], T. Quack[3], C. G. Grevelding[3], E. Roeb[2], L. M. R. Silva[3], C. Hermosilla[3], A. Taubert[3], B. Spengler[1]

[1]Institute of Inorganic and Analytical Chemistry, Justus-Liebig-University Giessen, Analytical Chemistry, Gießen, Germany
[2]Department of Gastroenterology, Justus Liebig University Giessen, Gießen, Germany
[3]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany

Introduction

Parasite-host interaction is on a mutual relationship: Parasites depend on nutrients from their host, and the host organism fights against exploiting its resources, leading to inflammation. Both effects affect the lipid composition and distribution.

High-resolution atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI) is a powerful tool to localize and identify lipids in biological samples.

Objectives

We aimed to visualize the impact of parasitic infection on the lipid profile of host tissue. We studied two different parasite-host models: *Besnoitia besnoiti*, forming cysts in bovine skin, and *Schistosoma mansoni* eggs, inducing granuloma formation in livers of hamsters as infection models.

Materials & Methods

AP-SMALDI MSI was performed on an AP-SMALDI5-AF ion source (TransMIT GmbH, Giessen, Germany) coupled to a high-resolution orbital trapping mass spectrometer (Q Exactive, Thermo Fisher Scientific, Bremen, Germany), enabling high lateral resolution (≥5 um pixel size) to depict fine structures in the tissue and high mass accuracy (resolution R=240000 at *m/z* 200) to analyze hundreds of biomolecules in parallel.

Cryosections (20 µm thick) of tissue samples were prepared using a cryomicrotome (HM 525, Thermo Fisher Scientific) and coated with matrix using a pneumatic sprayer (SMALDIprep, TransMIT GmbH) before analysis.

Data were uploaded to the Metaspace platform, and analytes were annotated using LipidMaps. Statistical analyses led to a list of up- and down-regulated compounds. MS images of these ions were created, enabling manual assignment to specific, parasite-induced structures (e.g., cysts, granuloma).

Results

We found lipids characteristic for infection in parasite-host systems.

For *S. mansoni*, specific lipids were found to be up- or downregulated in granuloma regions around the eggs. We even visualized very small features, such as *Schistosoma* eggs, inside the granuloma. Additionally, substructures within the granulomas were identified based on lipids with characteristic distributions.

For *B. besnoitia*, specific lipids for cyst walls and cyst content were identified, resulting in mass spectrometric images resembling H&E stained sections.

Conclusion

Using MSI, we analyzed parasites inside their host. This opens the door for future studies of different parasite-host systems, comparison of parasitic life stages, and analyzing drug-treated infected samples.

A123

Interactions of *Trypanosoma cruzi* and triatomines and intestinal bacteria: open questions

G. Schaub[1]

[1]Ruhr-University Bochum, Zoology/Parasitology, Raum NCDF 06/693, Fach Nr. 41, Bochum, Germany

Trypanosoma cruzi is the etiologic agent of Chagas disease, a neglected disease endemic in Latin America. If infected people are emigrating, it is also relevant to other countries. Analyses of the interactions of *T. cruzi* with the triatomines and the microbiota of the latter aim to interrupt the transmission cycle.

Investigations of the initial development of different strains of *T. cruzi* in the stomach differ whether or not all ingested blood trypomastigotes are killed there and allowing a development only those parasites which "escaped" into the small intestine. Investigating in vitro the interactions of *T. cruzi* with components of the small intestine, the fragility of the parasites should be considered to avoid damages of the surface by high centrifugation forces and protein-free buffers. Focussing on the development of epimastigotes in the rectum, the compounds at the hydrophobic attachment zone of the flagellum remain to be identified. Investigations of in vitro metacyclogenesis use conditions which are not occurring in the rectum of the vector. Therefore, concentrations of oxygen, monosaccharides, free amino acids, proteins and lipids should be determined. *T. cruzi* induces the synthesis of intestinal antibacterial compounds, and after a knockout of these compounds more bacteria and less *T. cruzi* develop. However, the strong diversity of the bacteria in the gut requires very detailed analyses. Mutualistic symbionts are only known for four species triatomines. The factors enabling a strong development of mutualistic symbionts in the stomach possessing a high level of antibacterial activity and the reduction of the population of these symbionts in the small intestine possessing a low antibacterial activity are important open questions. Also the vitamin B hypothesis requires attention. So far all mutualistic symbionts are Actinomycetales, and perhaps their unique compounds, mycolic acids, are important for the triatomines. Effects of *T. cruzi* on triatomines seem to occur in some parasite vector systems, but in experimental infections the supply with the mutualistic symbiont should be guaranteed, and feeding triatomines on living hosts gives an impression of the relevance of observed effects in populations at the field. The proteomic map of *T. cruzi*, the transcriptome and genome of the triatomine *Rhodnius prolixus* and the genome sequence of the symbiont *Rhodococcus rhodnii* should be evaluated for compounds relevant for the interactions in these systems.

A124

Abundance of *Ixodes ricinus* and influencing factors in northern Germany during 2021-2022

A. Springer[1], O. Bozbey[1], J. Probst[1], A. Topp[1], I. Bulling[2], M. Komorek[2], M. Komorek[2], O. Kahl[2], C. Strube[1]

[1]University of Veterinary Medicine Hannover, Institute for Parasitology, Centre for Infection Medicine, Hannover, Germany
[2]tick-radar GmbH, Berlin, Germany

Ixodes ricinus is the main vector of tick-borne pathogens in Europe. Although there have been many studies on this tick species, the driving factors of its local abundance are not yet fully understood. In northern Germany, tick abundance has been little studied, as compared to southern parts of the country. In this ongoing study, we aim to assess questing tick abundance and ecological correlates across northern Germany. From April to July of 2021 and 2022, i.e. during the main *I. ricinus* activity period, 43 sites in 13 areas were sampled monthly by the flagging method. Associations with habitat characteristics and climate data were analysed via mixed modelling. Approximately 25,000 *I. ricinus/inopinatus* specimens were collected. The average density of questing nymphs ranged from 0.9/100 m² to 227.4/100 m² depending on the studied site, with a mean number of adult ticks from 0.4 to 29.5/100 m². Altogether, higher questing tick densities were observed in 2021 (ø 1.0-359.0 nymphs/100 m²) than in 2022 (ø 0.8-149.3 nymphs/100 m²), but there were differences among areas and sites. Significant correlations of nymph density with forest type, temperature and precipitation were observed. The number of frost days in the preceding winter was significantly associated with nymph density in broadleaved and coniferous, but not in mixed and inner-urban forests. Monitoring of these comparatively high questing tick densities will be continued in this so far neglected geographic area in 2023.

A125

A global meta-analysis on mosquito host-feeding patterns

M. L. Wehmeyer[1], M. J. Tolsá García[2], J. Schmidt-Chanasit[1,3], D. Roiz[4], R. Lühken[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]Institut de Recherche pour le Développement México,, Mexico City, Mexico
[3]Universität Hamburg, Faculty of Mathematics, Informatics and Natural Sciences, Hamburg, Germany
[4]MIVEGEC, Université de Montpellier, IRD, CNRS, Montpellier, France

Mosquito host-feeding patterns are an important factor shaping the mosquito's vector capacity.As the interaction between vectors and hosts determine transmission cycles and risk of pathogen spill over, the understanding of host selection is important to assess the risk for human and animal health. Host selection can depend on intrinsic (e.g. mosquito genetics) as well as extrinsic factors (e.g. host availability). For example, anthropophagic mosquitoes are potential vectors for pathogens transmitted between humans (e.g. chikungunya virus), while opportunistically feeding mosquitoes can serve as bridge vectors for zoonotic viruses (e.g. West Nile virus).

In order to investigate mosquito host-feeding patterns, we collected the data from 339 scientific publications, covering a timeframe of nearly eight decades (1942-2019). We included studies, which sampled engorged mosquito females and screened the bloodmeal for hosts using any serological or molecular biological method. The collected and standardized parameters comprised mosquito species, blood meal hosts, collection method, method for blood meal analysis, time and date, and, if provided, land use and landscape information per study. These data on 544.809 identified blood meals of mosquitoes of 475 taxa allow a wide range of in-depth analysis of the host-feeding ecology of mosquitoes. For example, 285 of the taxa (60%) fed on humans, making them potential vectors of pathogens relevant for public health. Furthermore, the data indicate different host-feeding patterns: while some mosquito species like *Culex quinquefasciatus* show a broad host range, clear preferences for non-human mammalian species are evident for *Culex tritaeniorhynchus*. Additionally, the present dataset can be used for standardized classifications of general host-feeding patterns. This comprehensive meta-analysis helps to unterstand the interaction between mosquito and host species to understand global transmission patterns of mosquito-borne pathogens.

A126

Novel approaches to repel insect vectors

N. Verhulst[1], *P. Brok*[1], *A. Hochstrasser*[1], *A. Mathis*[1]

[1]Laboratory of Parasitology, Vetsuisse Faculty, Zürich, Switzerland

Introduction

The use of repellents can reduce the number of insect bites and thereby the risk of pathogen transmission. However, current repellents lack effectiveness as they only work for a limited time and must be applied directly on the skin. Biting midges (*Culicoides* spp.) are of major significance as vectors of veterinary pathogens. Like mosquitoes, they are attracted to their hosts by carbon dioxide in exhaled air and by their body odours, which are mainly produced by skin bacteria. This opens the possibility to protect animals from biting midges by supplementing the skin microbiome with probiotics that reduce their attractiveness. As another option to this probiotics approach, spatial repellents may be convenient as they eliminate the need to apply repellents directly onto the skin.

Objectives

Identify skin bacteria from sheep and spatial repellents that reduce biting by midges.

Materials & Methods

The skin bacterial composition of 89 adult female sheep from different breeds was determined by metabarcoding. Skin bacteria were cultivated on artificial media, and the attractiveness of selected isolates to biting midges determined in laboratory settings using a Y-tube olfactometer and membrane feeding assays. In addition, spatial repellency of different compounds was determined in a high-throughput tube setup and in a cage setup with automated tracking of the biting midges.

Results

We were able to detect repellent effects and a significant reduction in feeding rate of some of the skin bacteria tested. Next, a repellent bacterial strain will be applied on sheep in an exploratory study. Some of the tested spatial repellents were as effective as the golden standard DEET in the high-throughput tube setup. In addition, spatial repellency was observed in the cage setup and visualized by tracking and heatmaps. These spatial repellents will be tested in large scale semi-field experiments.

Conclusion

Applying skin probiotics could be a novel solution for long-lasting protection against biting insects, in contrast to the topical application of repellents that evaporate within hours, and spatial repellents could protect from a distance. These studies show that novel approaches to repel insect vectors may help to protect animals and humans against insect bites and thereby reduce pathogen transmission.

Funding: Gebert Rüf Stiftung (GRS-089/20); FAN UZH & Gebauer Stiftung; Federal Food Safety and Veterinary Office.

A127

The *Anopheles maculipennis* complex in the Republic of Moldova: geographical distribution, host feeding preferences and species composition with first record of *Anopheles daciae*

T. Şulescо[1,2], J. Schmidt-Chanasit[1,3], F. G. Sauer[1], R. Lühken[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Department of Arbovirology, Hamburg, Germany
[2]Institute of Zoology, Laboratory of Entomology, Chisinau, Moldova
[3]Universität Hamburg, Faculty of Mathematics, Informatics and Natural Sciences, Hamburg, Germany

Introduction. The *Anopheles maculipennis* complex includes the main vectors of malaria parasites in historic Europe. Besides, *A. maculipennis* s.l. has been found to be naturally infected with Batai virus, West Nile virus, Usutu virus and filarial nematodes. However, data on the spatial distribution of different species are outdated or limited for many European countries. Research on *Anopheles* species was mainly neglected after malaria eradication in Moldova about 70 years ago and only fragmented data were obtained during the monitoring of other mosquito species.

Objectives. The aim of this study was to update the data on the occurrence, distribution and host feeding preferences of the *A. maculipennis* complex members in Moldova using molecular screening techniques.

Materials and methods. The field work was conducted in 2016, 2017 and 2019 in 70 locations in Moldova. *A. maculipennis* s.l. females were sampled using CO2 - baited CDC traps, CDC light traps and mouth aspirators inside and outside animal stables and natural caves. Species-typing was conducted with PCRs targeting the ITS2-gene. The cytochrome *b* gene and 16S gene-fragments were sequenced to identify the vertebrate host in mosquito blood meals. Distribution maps were constructed in combination with previous studies and georeferenced records from historic literature.

Results. Four taxa of the *A. maculipennis* complex were identified from 297 specimens: *A. atroparvus*, *A. daciae*, *A. maculipennis* s.s. and *A. messeae*. *Anopheles daciae* was recorded for the first time in Moldova. The most widely distributed and abundant species was *A. maculipennis* s.s. The distribution of *A. daciae* and *A. messeae* overlapped in southern Moldova. Overall, 158 DNA sequences of the cytochrome *b* gene and 16S gene have been obtained from the engorged mosquitoes. Cattle (134 specimens, 84.8 %) and pig (10 specimens, 6.3 %) were the most commonly found hosts, followed by human (4 specimens, 2.5 %) dog (4 specimens, 2.5 %), horse (3 specimens, 1.9 %) and chicken (2 specimens, 1.3 %). *Anopheles daciae* (72 specimens, 24.3 %) was found to have the highest host diversity including cattle, poultry and humans.

Conclusion. The presence and distribution range of *A. atroparvus*, *A. maculipennis* s.s. and *A. messeae* in Moldova was confirmed for the first time by molecular sequencing. *Anopheles daciae* is a new record for the country. The *A. maculipennis* complex was found to have a high host diversity with prevalence of non-human mammals.

A128

Avian *Haemosporida* in mosquitoes collected in Germany

K. Köchling[1], G. Schaub[2], D. Werner[3], H. Kampen[1]

[1]Friedrich-Loeffler-Institut, Institute of Infectology, Greifswald, Germany
[2]Ruhr-University Bochum, Zoology/Parasitology, Bochum, Germany
[3]Leibniz-Centre for Agricultural Landscape Research, Müncheberg, Germany

Avian Haemosporida are a diverse group of protozoan blood parasites found worldwide, except at the poles. In Germany, several studies have demonstrated high infection prevalences with the haemosporidian genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* in birds, but little information exists about their specific native vectors. Mosquitoes are the main vectors of avian *Plasmodium*-species, which are responsible for the deaths of numerous exotic (= immunologically naïve) birds in zoos, every year, while native birds are adapted to the parasites and largely protected by an effective immune response. In the presented study, mosquitoes were trapped in bird/animal parks and riverine floodplains, and aspirated in gardens from 2020 to 2022. Mosquito females were pooled with up to 10 specimens according to species, location and date. Extracted DNA was screened for avian Haemosporida-specific mitochondrial rDNA using a realtime-PCR. Positive samples were amplified by a *Plasmodium*/*Haemoproteus*-specific nested PCR targeting the partial cytochrome b gene for species identification. Of 2,632 pools with 8,841 female mosquitoes, reactions of 47 pools confirmed an infection. The results show the occurrence of *H. majoris* (n = 1) as well as several *Plasmodium* species: *P. relictum* (n = 17), *P. matutinum* (n = 13), *P. vaughani* (n = 10), *P. circumflexum* (n = 3), *P. cathemerium* (n = 1) and *Plasmodium* sp. (n = 2). The parasites were detected in three different mosquito species groups: *Cx. pipiens* s.l. (n = 40), *Cs. morsitans/fumipennis* (n = 6) and *Ae. cinereus/geminus* (n = 1). Although the overall pool infection prevalence (1.8 %) appears to be low compared to a study from neighbouring Austria (6.4 %), the minimum infection rate is higher in the present study (5.3 vs. 3.7 in Austria). We not only demonstrated the ongoing circulation of *Plasmodium* and *Haemoproteus* parasites in the German mosquito population but show, for the first time, the presence of *P. cathemerium* in field-collected mosquitoes in Germany.

A129

Objective *in vitro* drug testing for *Echinococcus granulosus*

M. Kaethner[1,2], M. Preza[1], T. Kämpfer[1,2], P. Zumstein[1], C. Tamponi[2], A. Varcasia[2], A. Hemphill[1], K. Brehm[3], B. Lundström-Stadelmann[1,4]

[1]University of Bern, Institute of Parasitology, Vetsuisse Faculty, Bern, Switzerland
[2]University of Bern, Graduate School for Cellular and Biomedical Sciences, Bern, Switzerland
[3]University of Würzburg, Institute of Hygiene and Microbiology, Würzburg, Germany
[4]University of Bern, Multidisciplinary Center for Infectious Diseases, Bern, Switzerland

Introduction

Cestodes of the genus *Echinococcus* cause the zoonotic diseases alveolar echinococcosis (AE) and cystic echinococcosis (CE). An *in vitro* drug screening pipeline is established for AE, but drug efficacy assessments against *E. granulosus* are mainly performed via subjective eosin exclusion test on protoscoleces (PSCs), which are not the disease-causing stage. Thus, there is the urgent need for unbiased drug tests against *E. granulosus*.

Objectives

Our objectives were to validate whether the current *in vitro* drug screening assays for *E. multilocularis* metacestodes, protoscoleces and primary cells can be applied to *E. granulosus*. Furthermore, we aimed to establish *E. granulosus* metacestodes cultures that can be maintained *in vitro* for prolonged periods of time and to investigate whether proliferative primary cell cultures of *E. granulosus* could form new metacestode vesicles.

Material and methods

We applied an *in vitro* screening cascade established for *E. multilocularis* to *E. granulosus* to compare the efficacy of several standard drugs (niclosamide, nitazoxanide, albendazole, monepantel, mefloquine, buparvaquone and MMV665807). Efficacy against metacestodes was measured by damage marker release and vesicle viability assays. Efficacy against PSCs was assessed by motility assay, and viability assay was employed to investigate the impact on stem cells. Proliferation of primary cells was assessed by EdU incorporation and metacestode vesicle formation assays.

Results

In vitro cultured *E. granulosus* metacestodes exhibited an intact laminated layer. Comparing drug efficacy against *E. multilocularis* and *E. granulosus in vitro*, the damage marker release assay and the metacestode viability assay showed similar drug responses for most tested drugs, while buparvaquone and MMV665807 showed significantly higher activity to *E. multilocularis* metacestodes. Albendazole had a higher impact on *E. granulosus* PSCs. Stem cell assays showed similar activities for most tested drugs, but nitazoxanide, albendazole and monepantel showed higher activity to *E. multilocularis* stem cells. *E. granulosus* primary cells were successfully cultured for four weeks, underwent proliferation and formed novel metacestodes.

Conclusion

Our results show that established drug screening assays can be applied to *E. granulosus* and that its stem cells can be cultured to form novel metacestodes *in vitro.* This allows *in vitro* screening of drugs against *E. granulosus* in an unbiased and objective manner.

A130

Efficient method of extracting off-host stages of *Tunga penetrans* (sand flea) for the identification of transmission hotspots

A. K. Matharu[1,2], P. Ouma[2], M. M. Njoroge[2], A. Hyuga[2], F. Mutebi[3], J. Krüken[1], H. Feldmeier[4], L. Elson[5], U. Fillinger[6]

[1]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany
[2]International Centre for Insect Physiology and Ecology, Human Health Department, Nairobi, Kenya
[3]Makerere University, College of Veterinary Medicine, Kampala, Uganda
[4]Charité – Universitätsmedizin Berlin, Neglected Tropical Diseases, Berlin, Germany
[5]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya
[6]International Centre for Insect Physiology and Ecology, Human Health Department, Homa Bay, Kenya

Introduction

Tunga penetrans, (sand flea) is the cause of a severely neglected parasitic skin disease in the tropics and received little attention from entomologists to understand the transmission ecology.

Objective

Like all fleas, *T. penetrans* has environmental off-host stages and we used the Berlese-Tullgren funnel method to extract these off-host stages from soil samples to identify the disease transmission hotspots within rural households in Uganda and Kenya.

Material and Methods

The Berlese-Tullgren Funnel extractor was developed from locally available material in a small size with several units to fit the purpose of our work. We calibrated the apparatus by investigating the influence of different bulb powers as heat source and two extraction times. Field surveys were conducted in 50 tungiasis affected households. A total of 65 outdoor samples and 160 indoor samples were collected and extracted. The odds ratios or rate ratios and their 95% confidence intervals were calculated using generalized models in R software.

Results

The impact of heat (bulb wattage) and time (hours) on the efficiency of extraction was demonstrated and through a stepwise approach standard operating conditions defined for parallel extraction of 80% (95% CI 71-87%) of all present off-host stages from any given soil sample alive. The odds of finding off-host stages from indoor samples associated with infected individuals sleeping places was 7-fold higher (95% CI 2.4-21.5) than finding them from outdoor samples and for every larva found in the outdoor environment, 29 larvae were found indoors (95% CI 7.7-110.2).

Conclusions

Our findings highlight that the indoor sleeping areas are the transmission hotspots for tungiasis and can be targeted for disease control and prevention measures.

A131

Efficacy of natural and synthetic insect growth regulators for the control of sand fleas (*Tunga penetrans*) in laboratory bioassays

A. K. Matharu[1,2], P. Ouma[1], J. Krüken[2], L. Elson[3,4], U. Fillinger[5]

[1]International Centre for Insect Physiology and Ecology, Human Health Department, Nairobi, Kenya
[2]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany
[3]KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya
[4]University of Oxford, Nuffield Department of Medicine, Oxford, United Kingdom
[5]International Centre for Insect Physiology and Ecology, Human Health Department, Homa Bay, Kenya

Introduction:*Tunga penetrans,* (sand flea) is the cause of a tungiasis, a severely neglected tropical parasitic skin disease. Even if the disease is treated, re-infection occurs almost immediately due to off-host stages. It is, therefore, vital to identify targets for parasite control as treatment and prevention tools. Insect growth regulators such as pryriproxyfen have the ability to mimic a natural hormone in insects and disrupt their growth and is used widely for parasite control in pets and livestock, and is available in low-income countries for plant pest control. Neem oil, a natural product available in many tropical regions, has similar properties on a range of insects.**Objective:**To implement dose-response bioassays under controlled laboratory conditions to assess the efficacy of both products against *Tunga* off-host stages and to establish the optimum concentration for field evaluations.**Material and Methods:**Dose-response tests with four increasing exposure amounts of neem and pyriproxyfen were implemented for their impact on off-host stage development and adult emergence. These experiments were replicated 10 times on different dates containing 10 larvae each from different collection batches.**Results:**Pyriproxyfen was confirmed as a powerful insect growth regulator. Whilst in the unexposed controls 100% of all larvae successfully developed into adults, having pupated at day 10 after collection and taking a median of 9.5 (IQR 4) days in the cocoon until adult emergence on day 30 (IQR 4) after collection. Pyriproxyfen prevented 100% of the larvae to pupate in as low concentrations as 0.0075 parts per million (ppm) active ingredients. Neem oil was only effective at relatively high dosages. The mode of action for the neem oil, was consistently different from pyriproxyfen since larvae died in the process of trying to pupate around 10-12 days after collection and out of the larvae that managed to pupate, pupation took place by day 10 as in the controls but only 30% managed to emerge as adults. However, the effective dose of 15 ppm for LD50 would translate in an application of 60 ml of neem oil per square metre of floor area in the field.**Conclusions**Pyriproxyfen-based products for spray application on infested household floors should be tested for potential disease control. The impact of neem oil was only achieved at extremely high application rates and hence would not be a feasible product to take forward.

A132

Population biology of *Strongyloides stercoralis* (nematoda) – How many different species and hosts are there?

V. Amarashinghe[1], S. Gyarteng[1], X. Yin[1], D. Harbecke[1], A. Streit[1]

[1]MPI für Biologie Tübingen, Integrative Evolutionsbiologie, Tübingen, Germany

The estimated number of people infected with *Strongyloides stercoralis*, the species which causes the vast majority of human strongyloidiasis cases, has been corrected upwards over the last few years and has now surpassed the 600 millions.

Recent studies by us and others revealed considerable genetic and genomic diversity and variability in the biology, in particular life cycle and host preferences, of *Strongyloides stercoralis (s.l)*. Therefore, it is questionable that what is currently considered to be *Strongyloides stercoralis* is really only one species.

In order to complement the previous studies, we are currently collecting *S. stercoralis* from human and animal (i.e. canine and non-human primate) hosts is Ghana and in Bangladesh. Results from these collection efforts and an update on the establishment of a collection of live S*trongyloides stercoralis* isolates will be presented.

A133

In Vitro Effect of Artemisinin and Its Derivatives on the Metabolic Processes of *Ascaris suum* Third Stage Larvae

Z. Kidiavai[1], A. Kundik[1], J. Krücken[2], S. Hartmann[1]

[1]Institute of Immunology, Veterinary Medicine, Berlin, Germany
[2]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany

Soil transmitted helminths (STH) infect a total of 807 million – 1.2 billion people in the world, *Ascaris lumbricoides*being the most prevalent gut nematode in sub-Saharan Africa. Important to note is that co-infection with malaria, caused by *Plasmodium falciparum,* and STH is common in areas with overlapping endemicity. WHO recommends the use of artemisinin-based combination therapy (ACTs) as the first-line treatment of symptomatic malaria.

Artemisinin and its derivatives; artemether, artesunate and dihydroartemisinin (ARTs), have pharmacological activity not only against *Plasmodium falciparum* but also helminths i.e., *schistosome spp.,* viruses i.e., SARs Cov-2, cancers like lung carcinoma and autoimmune diseases i.e., inflammatory bowel syndrome. Our study aims to investigate the in vitro antinematode activity of artemisinin, artesunate and artemether on the energy metabolism of *A. suum* L3.

A. suum L3 were exposed to artemisinin, artesunate or artemether for 24 hours and evaluated for general metabolic activity or ATP production using the resazurin assay or bioluminescence ATP assay respectively. We detect a dose dependent inhibition by artesunate and artemether on the metabolic activity of *A. suum* L3. Thus, the derivatives, artemether and artesunate, were more effective when compared to artemisinin. Resazurin has been reported to detect mitochondrial function, therefore, a potential mechanism of action of artesunate and artemether could be an interference with the electron transport chain of the mitochondria. Consequently, fluorescence lifetime imaging microscopy is used in current experiments to further investigate metabolic enzymes affected in artesunate and artemether exposed *A. suum* L3. Thus far, our data indicate that ARTs may have a direct inhibitory effect on the nematode metabolism.

A134

Identification of a novel anti-schistosomal multi-stage Component X derived from mouse serum

J. Ertl[1], F. U. Prodjinotho[1], Y. Hamway[1], S. Häberlein[2], C. G. Grevelding[2], M. Haselbeck[3], C. Prazeres da costa[1]

[1]Technische Universität München, Institut für Mikrobiologie, Immunologie und Hygiene, Munich, Germany
[2]Justus-Liebig-Universität Gießen, Parasitologie, Gießen, Germany
[3]Technische Universität München, Chair of Biotechnology, Munich, Germany

Praziquantel (PZQ) is the drug of choice for the treatment and transmission control of schistosomiasis, the most important so-called neglected tropical disease (NTD). PZQ solely targets the adult worms but is ineffective on early migrating larval stages and, thus, does not protect from reinfection. The widespread use of PZQ favors the risk of the development and spread of drug resistance. There is a need to develop novel drugs effective against immature, juvenile, and adult worm stages. We have recently demonstrated the presence of not yet identified soluble multi-stage schistosomicidal factors in the serum of mice, the most widely used experimental laboratory host. The present study aimed to build on these findings to identify the active compound(s) in mouse serum and characterize the anti-schistosome properties.

We used large-scale fractionation of mouse serum to investigate the susceptibility of all schistosome stages in our novel in vitro culture platform that supports long-term larval survival and development. Subsequent comparative mass spectrometry analysis revealed a set of candidates we selected based on enzymatic properties and screened for their multi-stage schistosomicidal efficacy. The hit compound mode of action was defined via confocal microscopy.

Through in vitro screening, we observed that a phospholipase which we named Component X shared a similar schistosome-killing phenotype with mouse serum. Component X lethally affected parasites at all stages, including ex vivo adult worms, in a concentration- and time-dependent manner and is upregulated in infected animals compared to controls. Moreover, the treatment with sublethal doses of Component X affected tegument integrity and disrupted lipid layers and the structure of the parasite"s reproductive system.

Overall, our data reveal that Component X represents a multi-stage schistosomicidal host component, essentially produced in response to infection which could potentially be explored further for the development of novel therapeutic strategies.

Fig. 1

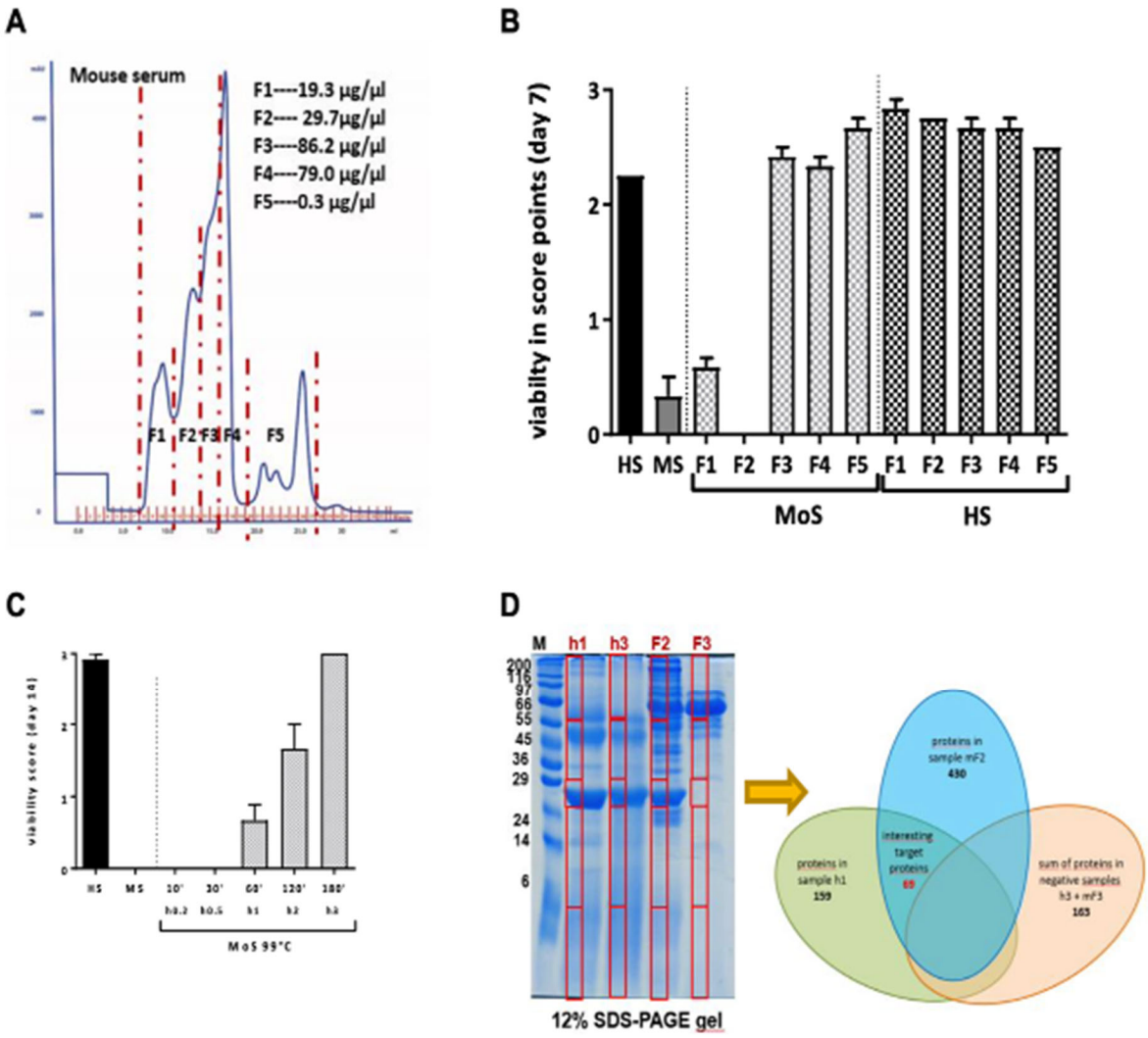
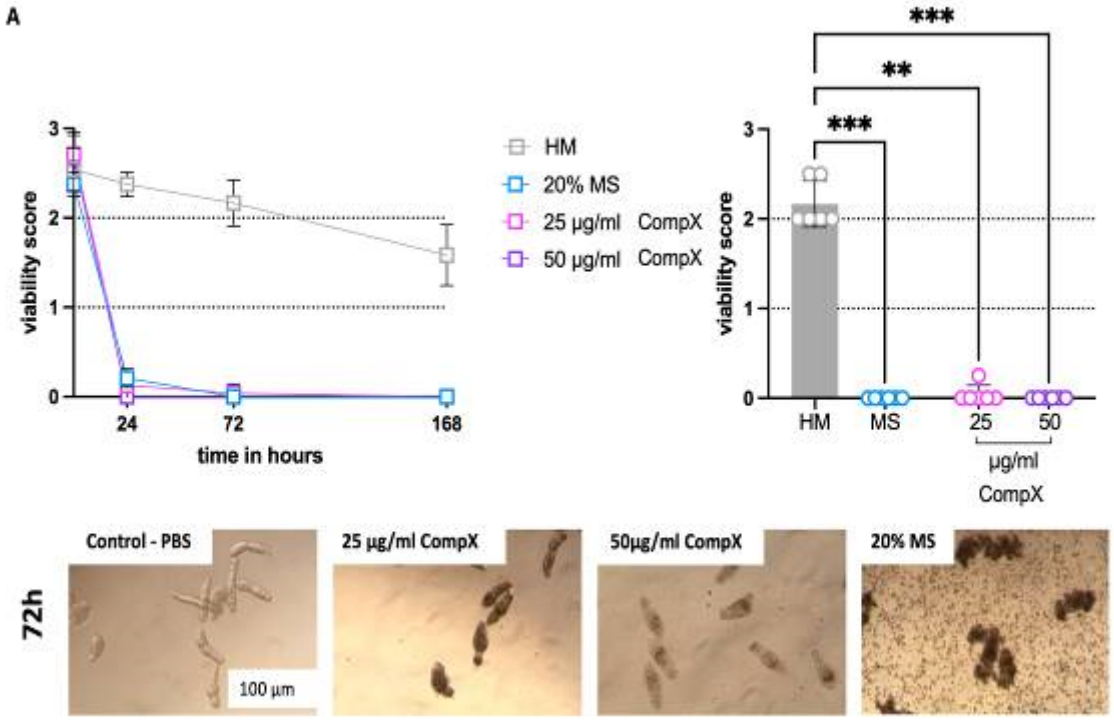

A
Mouse serum
F1----19.3 µg/µl
F2---- 29.7µg/µl
F3----86.2 µg/µl
F4----79.0 µg/µl
F5----0.3 µg/µl
F1
F2
F3
F4
F5
B
viability in score points (day 7)
HS
MS
F1
F2
F3
F4
F5
F1
F2
F3
F4
F5
MoS
HS
C
viability score (day 14)
HS
MS
10'
30'
60'
120'
180'
h0.2
h0.5
h1
h2
h3
MoS 99°C
D
M
h1
h3
F2
F3
200
116
97
66
55
45
36
29
24
14
6
12% SDS-PAGE gel
proteins in sample mF2
430
interesting target proteins
69
proteins in sample h1
159
sum of proteins in negative samples h3 + mF3
163

Fig. 2


A
viability score
time in hours
24
72
168
HM
20% MS
25 µg/ml CompX
50 µg/ml CompX
***
**
***
HM
MS
25
50
µg/ml
CompX
72h
Control - PBS
100 µm
25 µg/ml CompX
50µg/ml CompX
20% MS

A135

Targeting *Schistosoma mansoni* microRNA with small molecules to modulate host-parasite interactions

Y. Hamway[1], K. Zimmermann[2], M. J. J. Blommers[2], C. Häberli[3], S. Kulkarni[4], S. Skalicky[5], M. Hackl[5], M. Götte[2], J. Keiser[3], C. Prazeres da costa[1], T. Spangenberg[6], K. Azzaoui[2]

[1]Technical University Munich (TUM), Institute for Medical Microbiology, Immunology and Hygiene, Munich, Germany
[2]Saverna Therapeutics AG, Allschwil, Switzerland
[3]Swiss Tropical and Public Health Institute, Department of Medical Parasitology and Infection Biology, Allschwil, Switzerland
[4]EMD Serono Research & Development Institute, Inc., Billerica, MA, United States
[5]TAmiRNA GmbH, Vienna, Austria
[6]Ares Trading S.A., a subsidiary of Merck KGaA, Global Health Institute of Merck, Darmstadt, Germany

Introduction

Schistosoma is a genus of parasitic trematode responsible for causing schistosomiasis, this is perpetuated by an often chronic infection in which the parasite can persist for decades in the host. This persistence suggests a complex interaction where host factors are utilized for parasite development and the parasite modulates the host immune response[1]. One mechanism of immune modulation is through the use of microRNAs present in extracellular vesicles secreted by the parasites to regulate host immune cells. *Schistosoma* species *mansoni*, *japonicum* and *hematobium*, each produce a number of different microRNAs; with *S. mansoni* alone producing over 200 of these[2]. sma-miRNA-10 in particular has been shown to influence host T-cell fate determination through manipulation of the NF-κB pathway[3].

Objectives

Our objective was to identify small molecules that can bind *Schistosoma* miRNA and interfere with this immunomodulation.

Material & methods

We used a fragment-based screening approach by means of NMR to identify binders to the parasite microRNA-10. The small fragments identified were used to make larger elaborated molecules, which were then tested in cellular assays to confirm their ability to interfere with the action of sma-miR-10.

Results

We have identified low molecular weight tool compounds that can interfere with this microRNA-mediated *Schistosoma mansoni* manipulation of the host immune system. These molecules were demonstrated in cellular assays to counteract NF-κB activity inhibition by *S. mansoni* miRNA-10.

Conclusions

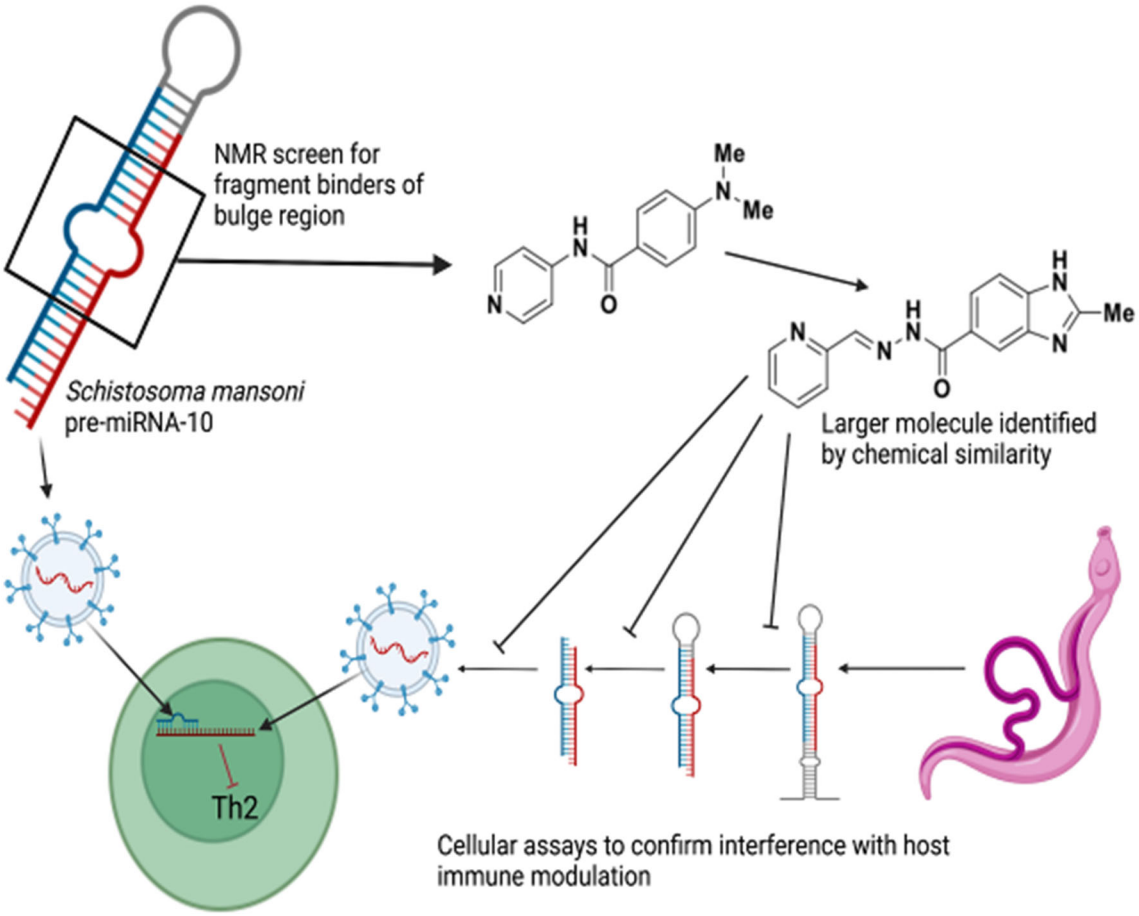
This work suggests a potential role for such compounds in modulating host-pathogen interactions. More generally, these compounds offer a proof-of-concept as microRNA-targeting small molecules, that yield a distinct phenotype.

[1] Y. Ofir-Birin, N. Regev-Rudzki, *Science* **2019**, *363*, 817-818.

[2] L. Zhu, J. Liu, G. Cheng, *Front Cell Infect Microbiol* **2014**, *4*, 165-165.

[3] T. Meningher, Y. Barsheshet, Y. Ofir-Birin, D. Gold, B. Brant, E. Dekel, Y. Sidi, E. Schwartz, N. Regev-Rudzki, O. Avni, D. Avni, *EMBO reports* **2020**, *21*.

Fig. 1



A136

Treatment efficacy of oxfendazole and flubendazole against the rodent filaria *Litomosoides sigmodontis* is dependent on the immune system

F. Risch[1], J. Scheunemann[1], J. Reichwald[1], B. Lenz[1], A. Ehrens[1,2], M. Koschel[1], A. Hörauf[1,2], M. P. Hübner[1,2]

[1]University Hospital Bonn, Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany
[2]German Center for Infection Research (DZIF), Partner site, Cologne/Bonn , Bonn, Germany

Introduction

Filarial worms can cause debilitating diseases such as lymphatic filariasis and onchocerciasis. Control of both diseases occurs mainly via mass drug administration (MDA), but there are no drugs available that can eliminate the adult worms ("macrofilaricide") using a short term treatment. Oxfendazole is a promising macrofilaricidal candidate with an improved oral availability in comparison to flubendazole and under preparation for phase 2 clinical trials in filariasis patients. Both benzimidazoles were tested in the *Litomosoides sigmodontis* mouse model, a model well established for preclinical drug development. Interestingly, several anti-filarial drugs require higher concentrations *in vitro* compared to *in vivo* to eliminate filariae. One possible reason for this discrepancy is that treatment efficacy of anti-filarial compounds is supported by the immune system.

Objectives

The aim of this study was to investigate the role of the immune system during treatment with two potential macrofilaricidal drugs, oxfendazole and flubendazole, and explore the potential to boost treatment efficacy via stimulation of the immune system.

Materials & Methods

Wildtype (WT) BALB/c, eosinophil-deficient dblGATA, IL-4R/IL-5$^{-/-}$, antibody-deficient µMT and B, T and NK cell-deficient RAG2/IL-2Rγ$^{-/-}$ mice were infected with *L. sigmodontis*. Mice harbouring adult worms (35 dpi) were treated with an optimal and suboptimal dose of flubendazole (FBZ) or oxfendazole (OXF) for up to 5 days. In a second part, WT mice were treated for two to three days with a combination of a suboptimal dose of OXF and IL-4, IL-5 or IL-33.

Results

At 70 dpi, WT mice displayed a reduction of the mean adult worm burden by >94% compared to vehicle controls after both FBZ and OXF treatments. In contrast, treatment with either drug in knockout mice led to no reduction (RAG2/IL-2Rγ$^{-/-}$) or a markedly lower reduction of the worm burden (dblGATA, IL-4R/IL-5$^{-/-}$, µMT). The efficacy of a shortened treatment of OXF (-17.2% adult worms vs. vehicle) could be boosted to a 69.6% reduction via combination with IL-5, but not IL-4 or IL-33.

Conclusions

Our results suggest that various components of the immune system support the filaricidal effect of benzimidazoles *in vivo* and present an opportunity to boost treatment efficacy.

A137

Effects of different knock-down resistance polymorphisms on pyrethroid susceptibility in the stable fly *Stomoxys calcitrans* and deep amplicon sequencing to screen for resistance haplotypes on German dairy farms

R. Daher[1,2], S. Fiedler[1], B. Bauer[2], W. Weiher[1], S. Steuber[1], A. M. Nijhof[2,3], P. H. Clausen[2], J. Krücken[2]

[1]The Federal Office of Consumer Protection and Food Safety (BVL), Drug Resistance, Berlin, Germany
[2]Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin, Germany
[3]Freie Universität Berlin, Veterinary Centre for Resistance Research, Berlin, Germany

Introduction

Stable flies (*Stomoxys calcitrans*) are biting flies that disturb the well-being of livestock and severely affect their productivity. Excessive, decades-long use of pyrethroids against insects has resulted in widespread resistance, and we previously reported the presence of pyrethroid resistance in stable flies on farms in Brandenburg (BB). Pyrethroid resistance in stable flies has been associated with *kdr* (L1014F) and *kdr-his* (L1014H) mutations but not super-kdr (M918T) in the voltage-sensitive sodium channel (*vssc*) gene.

Objectives

This study aimed to examine the correlation between pyrethroid resistance and the *vssc* genotype (*kdr* and *kdr-his*) in stable flies and to screen German stable fly populations for *kdr* and *kdr-his* mutations.

Materials & methods

In 2018, stable flies were collected from four dairy farms in BB and reared over two generations in the laboratory. The LD_{50} values for deltamethrin were determined for each isolate by topical application and compared with a susceptible laboratory strain (MSD). Selected, phenotypically tested flies were genotyped using PCR amplification of specific alleles (PASA). Using pooled stable fly samples collected from 65 dairy farms in BB, Schleswig-Holstein, and Baden-Württemberg in 2019, deep amplicon sequencing of the *vssc* gene was used to investigate the frequency and diversity of resistance alleles.

Results

The four tested field populations were identified as being phenotypically resistant to deltamethrin, with LD_{50} values of 18.0, 19.6, 40.5 and 47.0 ng deltamethrin/fly, 47.5 to 123.6-fold higher than for MSD strain. PASA showed the presence of *kdr* and *kdr-his* genotypes in all field populations, with significant ($p < 0.05$) higher odds to survive treatment already in heterozygous individuals compared to wild-type flies, and stronger effects of *kdr* than *kdr-his* on the resistance phenotype. Deep amplicon sequencing revealed the presence of *kdr* and *kdr-his* variants on 100% of the farms with up to 55% of *kdr* and 85% of *kdr-his* alleles on individual farms. Phylogenetic analysis suggested at least two independent origins of both, kdr and kdr-his variants. In 19/65 pools, the world-wide first evidence for *super-kdr* alleles in stable flies (frequency 0.6-9.5%) was observed.

Conclusion

Pyrethroid resistance genotypes are widespread in stable fly populations on German dairy farms. The deep amplicon sequencing assays will allow to screen for pyrethroid resistance markers on a nation-level scale.

A138

The value of *P. falciparum* field isolates for the prioritization of drug combinations to derisk malarial drug development: the case of Cabamiquine (M5717) – Pyronaridine

M. Maiga[1], S. Wicha[2], T. Spangenberg[3], L. Dembélé[4], C. Demarta Gatsi[3]

[1]Université des Sciences, des Techniques et des Technologies de Bamako, Bamako, Mali
[2]University of Hamburg, Department of Clinical Pharmacy, Hamburg, Germany
[3]Merck KGaA, Eysins, Switzerland
[4]Malaria Research and Training Centre, Bamako, Mali

Introduction

Development and spreading of drug-resistant parasites substantially threaten malaria control and elimination. New antimalarial combination drugs are needed and required mandatory preclinical studies to determine the optimal combination partner, such as *in vivo* mice models and *in vitro* checkerboard assays. These models have been developed with long adapted *P. falciparum* laboratory strains leading to genetic modification and deletion of essential pathways of the parasites The translational value of these models to patient isolates is questionable and may explain the discrepancies observed between research and clinical trial data. This study is the first attempt to use freshly-derived *P. falciparum* isolates from patient to develop an *in vitro* checkerboard assay to assess drug-drug interaction (DDIs) using a promising new antimalarial combination of cabamiquine (M57171) and pyronaridine, soon entering Phase II.

Methods

Concentration and time-dependent *in vitro* cidial activity of cabamiquine and pyronaridine alone and in combination were determined against *P. falciparum* field isolates using standard 48 hours SYBR Green assay combined with Mitotracker readout. *In vitro* data were analyzed using non-linear mixed effects modelling describing the parasite growth and killing kinetics.

Results

For the first time in malaria, we used *P. falciparum* field isolates to assess DDIs using *in vitro* checkerboard assays. With cabamiquine and pyronaridine as experimental model, we successfully generate a range of EC50"s of the two drugs either alone or in combination. Moreover, we highlighted the importance of the combination in controlling possible adaptive resistance to one of the two drugs. The data were validated by using reference laboratory strains.

Conclusions

Being the first time that DDIs were studies using *P. falciparum* isolates directly from patient to inform the dose rate and dosing regiments of cabamiquine and pyrodnaridine in Phase II and III, offering a unique opportunity to validate the relevance of this new model. These data highlight the power of using non-adapted strains to better understand the future efficacy, resistance profile and safety of new antimalarial combination. If clinically confirmed these data are highlighting the central role that cutting edge African research needs to play in malaria control and eradication.

A139

Proteomic characterization of *Toxoplasma gondii* ME49 derived strains resistant to the artemisinin derivatives artemiside and artemisone implies potential mode of action independent of ROS formation

J. Müller[1], C. Schlange[1], M. Heller[2], A. C. Uldry[2], S. Braga-Lagache[2], R. K. Haynes[3], A. Hemphill[1]

[1]Institute of Parasitology, University of Bern, Vetsuisse Faculty, Bern, Germany
[2]University of Bern, Proteomics and Mass Spectrometry Unit, Bern, Switzerland
[3]North-West University, Centre of Excellence for Pharmaceutical Sciences, Potchefstroom, South Africa

Introduction: The sesquiterpene lactone artemisinin and its amino-artemisinin derivatives artemiside (GC008) and artemisone (GC003) are potent antimalarials. The mode of action of artemisinins against *Plasmodium* sp is popularly ascribed to "activation" of the peroxide group by heme-Fe(II) or labile Fe(II) to generate C-radicals that alkylate parasite proteins. An alternative postulate is that artemisinins elicit formation of reactive oxygen species by interfering with flavin disulfide reductases resposible for maintaining intraparasitic redox homeostasis. Amino-artemisinins exhibit better activity against *Toxoplasma gondii* than artemisinin, however, the role of ROS has not been investigated.

Materials and methods: *T. gondii* tachyzoites were grown in human foreskin fibroblasts. The *T. gondii* reference strain ME49 was treated with stepwise increasing amounts of artemisone and artemiside, yielding the artemisone resistant strain GC003R and the artemiside resistant strain GC008R. The formation of reactive oxygen species (ROS) was assayed using dichlorofluorescein-diacetate (DCF-DA).

Results: The amino-artemisinins are effective *in vitro* against the non-heme-degrading *T. gondii* tachyzoites with IC 50 values of 50–70 nM, and induce distinct ultrastructural alterations. However, *T. gondii* strains readily adapted to increased concentrations (2.5 µM) of these two compounds within few days. Differential analyses of the proteomes of these resistant strains compared to the wildtype ME49 revealed that 215 proteins were significantly downregulated in artemisone resistant tachyzoites and only 8 proteins in artemiside resistant tachyzoites as compared to their wildtype. Two proteins, namely a hypothetical protein encoded by ORF TGME49_236950, and the rhoptry neck protein RON2 encoded by ORF TGME49_300100 were downregulated in both resistant strains. Eight proteins involved in ROS scavenging including catalase and superoxide dismutase were amongst the differentially downregulated proteins in the artemisone-resistant strain. In parallel, ROS formation was significantly enhanced in isolated tachyzoites from the artemisone resistant strain and – to a lesser extent – in tachyzoites from the artemiside resistant strain as compared to wildtype tachyzoites.

Conclusions: Amino-artemisinin derivatives display a mechanism of action in *T. gondii* distinct from *Plasmodium*, possibly linked to TGME49_236950, and the rhoptry neck protein RON2.

Fig. 1

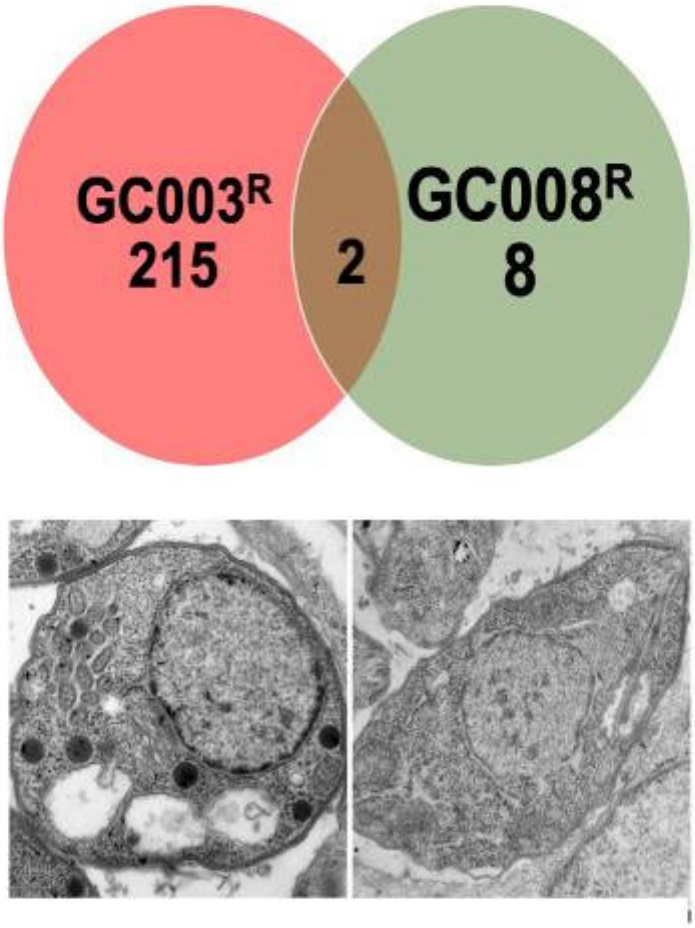
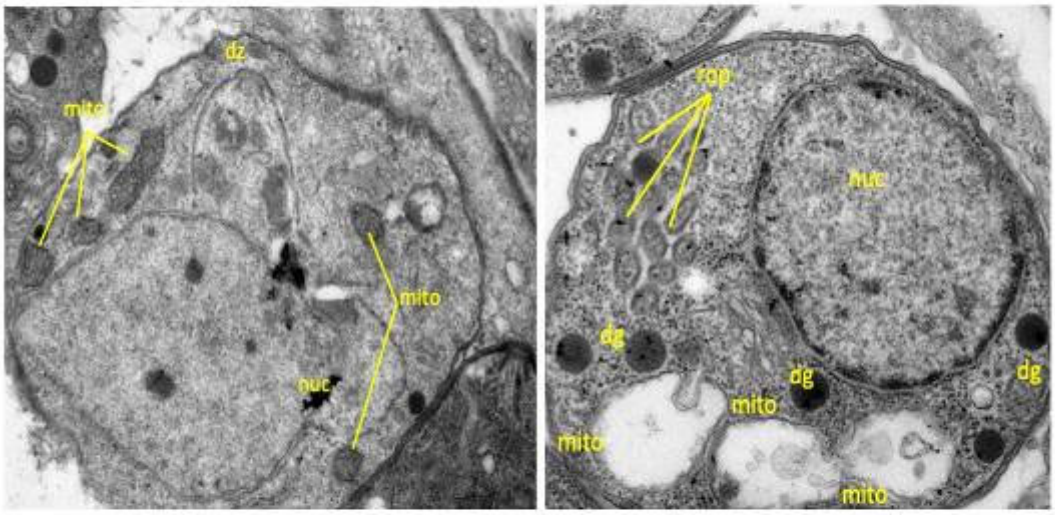

GC003R
215
2
GC008R
8

Fig. 2



A140

The mRNA decapping enzyme of *Trypanosoma brucei* is a promising drug target

S. Kramer[1], P. Castaneda Londono[1], M. Gorna[2], M. Zoltner[3]

[1]University of Würzburg, Cell and Developmental Biology , Würzburg, Germany
[2]University of Warsaw, Warsaw, Poland
[3]Charles University Prague, Prague, Czech Republic

Degradation of mRNAs is essential in all eukaryotes and contributes to the regulation of gene expression. The process usually starts with the removal of the poly(A) tail, is followed by the removal of the 5´ cap by a decapping complex and finishes by 5´-3´exoribonucleolytic decay. Kinetoplastids have conserved enzymes for the first and third reaction, but lack orthologues to all proteins of the decapping complex found in opisthokonts and plants. Instead, the Kinetoplastida decapping complex consists of the ApaH like phosphatase ALPH1 and several mostly Kinetoplastida-unique proteins: an example of convergent evolution.

ALPH1 is a promising drug target: it is essential in Kinetoplastida, the entire enzyme family of ApaH like phosphatases does not exist in mammals and the enzyme can be purified in an active and soluble form. Here, we present the development of a high throughput enzyme assay that we will use to screen a variety of available drug libraries.

POSTER PRESENTATIONS

P1

Adhesion forces and cellular mechano-transduction in parasitic flatworms

R. R. Nakate[1], J. Hörl[2], A. Mosig[3], F. Schacher[2], C. G. Grevelding[1], S. Häberlein[1]

[1]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany
[2]Friedrich-Schiller-Universität Jena, Institute of organic chemistry and Macromolecular chemistry and Jena center for soft matter, Jena, Germany
[3]Jena University Hospital, Institute of Biochemistry II, Jena, Germany

Introduction

"Physics of Parasitism" defines the physics and mechanics of parasites interacting with their hosts. Among the most prevalent and medically important groups of trematodes are schistosomes (blood flukes), which live in blood vessels while adult liver flukes live in the bile ducts of their final hosts. The species in focus, Schistosoma mansoni and Fasciola hepatica, exhibit remarkable features such as migratory juvenile stages, and occupation of different host niches (tissues) during their life. In addition, as typical for trematodes, these parasites have head and ventral suckers that aid migration and attachment processes. Although the regulation of biomechanical forces at organ (suckers) and cellular scales (mechanotransduction) is crucial for the survival and infection success of the parasites, these processes are little understood.

Objectives

This project investigates the physical forces occurring at the parasite-host interface by the action of suckers and the mechanisms of force transduction within the parasites. We will study whether adhesion forces differ between different fluke stages, sexes, and species, and whether these forces depend on the physical properties of the parasite"s environment, such as substrate stiffness and flow stress.

Materials & Methods

Traction force microscopy and fluorescence microscopy will be employed to quantify attachment forces of the parasites" suckers to different artificial host-like surfaces in vitro. Fluorescent hydrogels for traction-force measurements will be developed. Likewise, biochips for the culture of parasites under semi-natural conditions will be employed. Cellular proteins involved in mechanotransduction will be functionally analysed by RNA interference and chemical agonists or antagonists.

Results

Initial experiments have been conducted with different formulations of polyethylene glycol- and poly-acrylamide-based hydrogels. Culturing of the parasites in the biochips has been tested. To understand force transduction at a cellular level, mechanosensitive receptors and mechanoresponsive transcriptional regulator proteins have been identified and were successfully knocked down.

Conclusion

Fundamental knowledge on the biomechanics of the adhesion and the cell biology of parasitic flatworms will help to understand the remarkable adaptations of their "bauplan" to their parasitic lifestyle.

P2

Characterization of the binding-patterns underlying the cytoadhesion of *Plasmodium falciparum* infected erythrocytes

J. Allweier[1,2], J. Cronshagen[1], F. Kieferle[1], N. G. Metwally[1], J. Brehmer[1,2], T. Gutsmann[2], I. Bruchhaus[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Host Parasite Interaction, Hamburg, Germany
[2]Research Center Borstel, Biophysics, Borstel, Germany

Despite the advances in malaria control programs, malaria remains one of the most detrimental infectious diseases worldwide. In 2020, about 241 million cases of malaria were recorded, leading to 627,000 deaths. Infection with *Plasmodium falciparum* is the main cause of death among malaria patients. Therefore, developing new therapeutic strategies is needed to lower the burden of this infection.

A major difficulty in developing treatments and vaccines against *P. falciparum* malaria is the parasites" ability to switch the expression of antigens presented on the surface of infected erythrocytes (iEs). These antigens not only allow the iEs to evade recognition by the immune system but also to adhere to each other and the endothelium of blood vessels. Cytoadhesion is mediated primarily by members of the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) family, encoded by ap*pro*ximately 60 *var* genes per parasite genome. At least 24 endothelial cell receptors (ECRs) have been described as binding partners for iEs, including CD36, ICAM-1, and EPCR. *Pf*EMP1s are mutually exclusively expressed and randomly switched with a frequency of 0.025–3 % per replication cycle to evade detection by the immune system.

Until recently, this has been a major challenge in studying *Pf*EMP1-mediated cytoadhesion. We now established a method to generate transfectants that express only the *var* gene of interest and therefore present the corresponding *Pf*EMP1 population on the surface of iEs, through the selection-linked integration (SLI) approach. With a library of SLI-var transfectants, it is now possible to further characterize endothelial binding partners in static- and flow adhesion assays as well as using single cell force spectroscopy. Static binding assays with transgenic CHO cells presenting ECRs on the surface and with parasite transfectants presenting Var01 or Var16 have already led to the identification of the novel binding partners (e.g. TNFR2, VCAM-1, and P-selectin). The use of shear stress in an *in vitro* flow system has been shown to play a key role, as some receptor-ligand pairs form so-called slip-bonds, which only bind if shear force is applied. One such receptor might be ICAM-1, whereas binding to this receptor correlates with the development of severe malaria.

P3

N-glycomics analysis of *Anisakis* excretory/secretory products

I. Adduci[1], G. Cheng[2], K. Paschinger[3], S. Yan[1]

[1]University of Veterinary Medicine Vienna/Institute of Parasitology, Department of Pathobiology, Vienna, Austria
[2]Shanghai Tenth People's Hospital, Institute for Infectious Diseases and Vaccine Development, Tongji University School of Medicine, Shanghai, China
[3]Universität für Bodenkultur Wien, Department für Chemie, Vienna, Austria

Anisakis simplex is a parasitic aquatic nematode, which may cause mild-to-severe gastrointestinal allergic reactions (Anisakiasis) with clinical symptoms, such as rhinitis and urticaria in humans who accidentally consume raw or undercooked marine products contaminated with infective L3 *Anisakis* larvae. Several *Anisakis* excretory/secretory (ES) products and somatic proteins are known to be involved in IgE-mediated allergic reactions. In comparison to vertebrates, nematodes have a distinct machinery to glycosylate their proteins and unusual glycan structures have been reported previously, many of which play immunogenic and immunomodulatory roles in host-parasite interactions. While an early study indicated that O-glycans participate the cross-reactivity of antibodies in allergy patients to *A. simplex* somatic antigens, the N-glycosylation pattern of *Anisakis* and the potential role of N-glycans in allergic reactions remained unknown. The aim of this study was to characterise N-glycans from *Anisakis* ES products using mass spectrometry. We harvested ES products from larvae culture and released N-glycans from trypsinised proteins using PNGase A. Native glycans were pyridilaminated prior to HPLC separation and MALDI-TOF-MS/MS analysis. In addition, hydrofluoric acid and glycosidase digestions were performed to aid structural characterisation. MS data of 5h and 24h ES products indicated the presence of pauci-mannose and core fucosylated N-glycans as major species; tri-fucosylated and methylated glycans as well as complex-type and phosphorylcholine-substituted glycans were also detected. Our study provides the first insight into the N-glycosylation machinery of *Anisakis* and highlights the needs for investigating whether and which N-glycans are indubitably involved in the modulation of allergic responses.

Keywords: *Anisakis simplex*, parasitic nematodes, N-glycans, glycomics, allergens

P4

First molecular detection of *Neospora caninum* from naturally infected slaughtered camels in Tunisia

Y. Amdouni[1,2], I. Abedennebi[3], S. Amairia[2], A. Abdelkader[3], W. Chandoul[4], M. Gharbi[3]

[1]University of Bern, Institute of parasitology, Bern, Switzerland
[2]Laboratoire de Parasitologie, University Manouba, Institution de la Recherche et de l'Enseignement Supérieur Agricoles, École Nationale de Médecine Vétérinaire de Sidi Thabet,, Sidi Thabet, Tunisia
[3]Laboratoire d'anatomie Pathologique, École Nationale de Médecine Vétérinaire de Sidi Thabet, Sidi Thabet, Tunisia, Laboratoire d'anatomie Pathologique, École Nationale de Médecine Vétérinaire de Sidi Thabet, Sidi Thabet, Tunisia, Sidi Thabet, Tunisia
[4]Ministry of Agriculture, Water Resources and Maritime Fisheries, Arrondissement de Production Animale de Médenine, Médenine, Tunisia

Introduction: *Neospora caninum* has been documented to infect most domestic wildlife but is known to primarily infect dogs and cattle and is considered an important cause of abortion in camels.

Objective: The aim of this study was to estimate the molecular detection of *N. caninum* in tissues of naturally infected camelids.

Materials & methods: Brain, tongue (bottom and tip) and masseter muscles from 35 slaughtered camelids from Tataouine and Médenine regions were collected (n = 140 samples). PCR was used to amplify and detect *N. caninum* DNA in tissues samples followed by sequencing of some PCR products. A phylogenetic tree was then constructed to compare the partial sequences of the ITS1 gene with GenBank sequences. Histopathology examination was used to detect Neospora spp. cysts, but no lesions were observed.

Results: The overall molecular detection of *N. caninum* in camelids was 34.3% (12/35). The highest molecular detection of *N. caninum* was recorded in animals of more than 3 years old (6/9) and in animals aged between 1 and 3 years old (4/12).Whilst, the lowest molecular detection (2/14) was observed in animals 1 year or younger ($p = 0.035$). There were no significant differences in molecular detection of *N. caninum* according to both locality and gender ($p > 0.05$). Similarly, there was no difference of prevalence between different anatomical locations. Comparison of the partial sequences of the ITS1 gene revealed 100–95.5% similarity among our *N. caninum* amplicon (MW551566) and those deposited in GenBank.

Conclusion: These results highlight the presence of a risk infection by *N. caninum* in camels. For preventing *N. caninum* infection further studies are needed to improve our knowledge about the epidemiology of neosporosis in North Africa.

Key words: camelids, molecular detection, Neospora caninum, PCR, South Tunisia

P5

Comparative structural analysis of surface coat proteins in African trypanosomes

N. Aslam[1], *M. Engstler*[1], *N. G. Jones*[1]

[1]University of Würzburg, Chair of Zoology I - Cell and Developmental Biology, Würzburg, Germany

African trypanosomes are causative agents of Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) in sub-Saharan Africa. The cell surface of these trypanosomes is coated with a thick layer of prototypic, glycosylphosphatidylinositol-anchored proteins known as Variant Surface Glycoprotein (VSG), with a molecular size of ca. 60 kDa. The VSG coat acts to counteract the host immune response through antigenic variation by switching the expression between antigenically distinct VSGs from a repertoire of more than 1200 VSG genes. It also serves as a physical barrier to protect underlying invariant proteins from attacks by the host's immune system. In addition, directional swimming of the trypanosomes causes antibodies bound to the VSG coat to be dragged to the posterior pole of the trypanosome where they are endocytosed. The structures of VSGs in *Trypanosoma brucei* have been extensively studied, which consist of two or three domains, one N-terminal domain consisting of 350–400 residues, followed by one or two C-terminal domains each having 30–70 residues. The NTDs are divided into five main types (N1-N5) and CTDs are divided into six groups (Types 1-6). The VSGs in *T. congolense* and *T. vivax* are somewhat smaller, and are made up of a single domain that is similar to the *T. brucei* VSG N-terminal domain. Although the tertiary structure of NTDs of VSGs in *T. brucei* has been extensively studied, we have no structural information on either *T. congolense* or *T. vivax* VSGs, therefore, we are aiming to solve the structures of VSGs from these species. The first goal is to solve the structure of a number of different, selected *T. congolense* VSGs. The next step will focus on determining the structure of a *T. vivax* VSG. A genetically engineered cell line of *T. brucei* was constructed to express a *T. congolense* VSG, which was used to purify the VSG in its soluble form (sVSG). Purification of full-length sVSG was performed by anion exchange chromatography. An X-ray crystallography approach will be used to solve the structures of VSGs. Resolving the structural model of VSGs in *T. congolense* and *T. vivax* will provide a better understanding of the overall architecture and structure-function relationship of the surface coat in these species.

P6

Characterization of the surface lipidome of *Trypanosoma brucei*

K. Bongers[1], *T. Müller*[1], *M. Engstler*[1]

[1]University of Würzburg, Department of cell and developmental biology, Würzburg, Germany

Introduction

Trypanosoma brucei is a eukaryotic parasite. Subspecies of the parasite can infect humans and livestock. The parasites are transmitted by the tsetse fly. After infection of a mammal, they inhabit the blood and diverse tissues, where the parasite features a dense coat of a glycosylphosphatidylinositol-anchored variant surface glycoprotein (VSG). The VSG coat shields invariant proteins and the plasma membrane from the host immune system. The density of the VSG coat is tightly controlled by the parasite.

Objectives

We want to deepen our understanding of cell surface processes and how they can function at the biophysical limit. Therefore, we are trying to artificially recreate the surface of the trypanosome. An artificial membrane will give us the option to control all aspects of the surface system to investigate the mechanisms behind the high density of the VSG coat, how both VSG structure and density effects diffusion on the parasite surface, the antigenic switch of one VSG type to another and the largely unknown behaviour of invariant surface proteins.

Materials and methods

We developed an artificial membrane system on glass. Phospholipids and natural VSG proteins are dissolved in detergents and reconstituted into protein-dense proteoliposomes, which are then fabricated into supported lipid bilayers. The diffusion behaviour of VSG in the bilayers is analysed with single-molecule fluorescence microscopy.

In the near future, we aim to replace the simple lipid bilayer with a chemically diverse and structurally functional membrane. Therefore, we purify and identify the surface lipidome of *T. brucei* and use the trypanosome-derived lipid composition to create an artificial, trypanosome-specific membrane system.

Results

We developed a process that routinely creates artificial membranes with a high protein density. The diffusion behaviour of VSG on our artificial membrane resembles the observed *in-vivo* diffusion coefficients of plasma-membrane anchored VSG. Early results show that we can separate the plasma membrane from inner membrane compartments to selectively purify membrane lipids for reconstitution into artificial lipid bilayers.

Conclusion

By combining our newly developed tools, we want to draw a detailed picture of the biophysical processes at the host-parasite interface.

P7

The *Plasmodium falciparum* CCCH zinc finger protein ZNF3 is an RNA-binding protein important for gametocyte maturation and transmission to the mosquito

A. Farrukh[1], V. J. Mahlich[1], U. Distler[2], S. Tenzer[2], G. Pradel[1], C. J. Ngwa[1]

[1]RWTH Aachen University, Division of Cellular and Applied Infection Biology, Aachen, Germany
[2]University Medical Centre of the Johannes-Gutenberg University, Institute of Immunology, Mainz, Germany

CCCH-type zinc finger proteins represent a family of RNA-binding proteins with regulatory functions in mRNA processing. Previously, we identified a *Plasmodium falciparum* CCCH-zinc finger protein, here named ZNF3 (PF3D7_0315600), which was significantly up-regulated in its transcript levels following treatment of gametocytes with the histone deacetylase inhibitor Trichostatin A. We now show that ZNF3 is mainly expressed in immature gametocytes and can here be detected in the cytoplasm and the nucleus. We have generated a *glmS*-based ZNF3 knockdown parasite line (ZNF3-KD) for phenotypic analyses. Cell-based assays revealed essential roles of ZNF3 in asexual blood stage replication, gametocyte maturation and exflagellation. BioID analyses identified mainly RNA-binding proteins as potential interaction partners of ZNF3 and several other interactors could be assigned to the translational repression complex of *Plasmodium* gametocytes. We have verified the binding of ZNF3 to selected putative interactors by protein-protein interaction assays. Our combined data suggest that ZNF3 is an RNA-binding protein with regulatory functions in transcript regulation crucial for male gametogenesis and hence human-to-mosquito transmission of *P. falciparum*.

P8

Functional analysis of male competence factors potentially involved in the male-female interaction of *Schistosoma mansoni*

S. Geetha[1], X. Li[1], O. Weth[1], S. Welsch[1], M. F. Möscheid[1], C. G. Grevelding[1]

[1]Institute for Parasitology, Justus Liebig University, Veterinary Medicine, Gießen, Germany

Introduction

Schistosoma mansoni is a parasite that causes schistosomiasis, a neglected tropical disease. Schistosomes are unique in their reproductive biology, because the female depends on a constant pairing contact with a male partner to achieve sexual maturation. Although there are reports on functional description of GPCRs and their role in metabolic pathways in vertebrates, mammals and plants, roles of GPCRs in invertebrates are not well studied.

Objective

Our research focuses on the functional characterization of genes coding for male competence factors that may regulate the sexual maturation of the female after pairing in *S. mansoni*. To this end, we identified pairing-dependently expressed GPCRs and neuropeptide genes (npps) that are upregulated in males and downregulated in females after pairing. To unravel GPCR-npp interaction, we established MALAR-Y2H system, which provided first evidence for matching GPCR-npp couples. Certain GPCR-npp interactions have already been identified in previous studies of our group.

Materials and Methods

Here, I studied the function of GPCR 21, which was downregulated by RNA interference (RNAi) experiments *in vitro*. The experiment was performed in pairing-experienced (bsF) and inexperienced females (ssF) followed by repairing. RNAi effects on pairing stability, egg production, motility, and attachment behavior were monitored as physiological parameters. In addition, the localization of the GPCR 21 transcript was investigated by whole mount *in situ* hybridization (WISH). Finally, the morphological changes with respect to knock down were analyzed using microscopy.

Results and Conclusion

A male and ssF-dominated transcript profile of GPCR 21 was identified and reconfirmed earlier by our group. WISH localized transcripts were seen in the anterior region and the gut area of adult worms. The RNAi-mediated knockdown efficiencies for bsF and ssF were 39% and 64%, respectively. An increased percentage in attachment was observed after repairing. In both the bsF and ssF groups, an increased percentage in attachment was observed after repairing as well as a reduction in motility over time of the experiment. I observed an additional negative effect on egg production in the bsF group. I plan to do further investigation on identifying the npp interaction partner of GPCR 21 and will perform FRET analysis for its confirmation. The efficiency of RNAi experiments will be improved by performing a double knockdown.

P9

The Geographical Distribution of Human Cutaneous and visceral Leishmaniasis in Iran

H. Hajjaran[1], R. Saberi[2], M. Fakhar[2], S. A. Hosseini[2], A. Borjian[1], M. Mohebali[1], S. Ghodrati[1]

[1]Tehran University of Medical Sciences, Medical Parasitology and Mycology, Tehran, Iran
[2]Toxoplasmosis Research Center, Communicable Diseases Institute, Iranian National Registry Center for Lophomoniasis and Toxoplasmosis, School of Medicine, Mazandaran University of Medical Sciences,, Sari, Iran

Introduction: Leishmaniasis is one of the most common vector-borne parasitic diseases in Iran.

Objective: We systematically searched all the studies, reports, and documentation related to species identification and geographical distribution of causative agents of cutaneous (CL) and visceral leishmaniasis (VL) in Iran.

Methods: International databases were systemically searched for English articles. Searches were performed from 1999 to 2019 (20 years).

Results: In total, 10,586 CL isolates were identified, with two causatives of ZCL (*L. major*, n = 6,714) and ACL (*L. tropica*, n = 3,872) being reported in 19 provinces across Iran. In contrast, 542 isolates for VL cases were determined in 11 provinces. The pooled frequency of causative agents of CL isolates was 67.3% for *L. major* and 32.1% for *L. tropica*. In addition, the pooled frequency of causative agents of VL isolates was 97.1% for *L. infantum* and 2.9% for *L. tropica*.

Conclusion: The findings of this study showed that the main causative agents of CL and VL in Iran are *L. major* and *L. infantum*, respectively. The current study provides valuable data to encourage and direct researchers as well as public health managers in the comprehensive leishmaniasis control and prevention planning in Iran. In the end, the issue of the *Leishmania* RNA virus has become an interesting topic. LRVs exist within many species of the *Leishmania* isolates. According to evidence, LRV has been detected in *L. major*, *L. tropica* and *L. infantum* in Iran.

Fig. 1

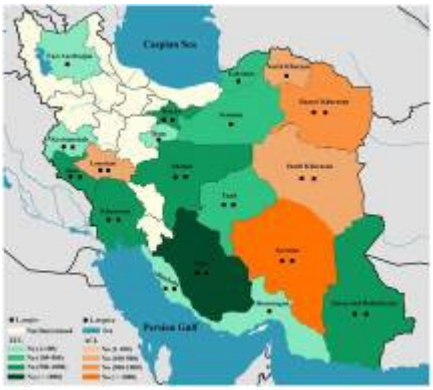


Fig. 2

P10

Endocytosis is required for access of surface-bound cargo to the flagellar pocket of *Trypanosoma brucei*

A. Konle[1], *B. Morriswood*[1]

[1]Universität Würzburg, Zell- und Entwicklungsbiologie , Würzburg, Germany

Trypanosoma brucei is an extracellular parasite which lives in the bloodstream of its mammalian host. The bloodstream is a nutrient-rich environment but also means that the parasite is in continuous exposure to the host immune system. To counter this threat, the parasite has developed specific survival strategies. Its surface is covered in a dense glycoprotein coat that is continually endo- and exocytosed to remove bound antibodies. Remarkably, all endo- and exocytosis is restricted to the flagellar pocket, a small invagination of the plasma membrane. It is not clear whether antibodies or cargo that are bound to the trypanosome surface passively enter the flagellar pocket, or if a more active mechanism is involved. The neck of the flagellar pocket has a number of distinct cytoskeleton-associated protein complexes coiled around its cytoplasmic face. One of these complexes is the hook complex, which contains the proteins TbMORN1 and TbSmee1. Knockdown of TbMORN1 results in impaired access of larger cargo to the flagellar pocket, suggesting a size exclusion limit. Uptake assays with different reporters revealed no change in the size limit after knockdown of TbMORN1 and TbSmee1, however. To test whether endocytosis was required for cargo entry to the flagellar pocket, the endocytic effector clathrin was knocked down. Clathrin knockdown phenocopied TbSmee1 knockdown, suggesting that endocytosis is required for the entry of surface-bound cargo into the flagellar pocket.

P11

Characterisation of egress-related proteins in *Plasmodium falciparum*

J. Küchenhoff[1], J. Sassmannshausen[1], S. Bennink[1], U. Distler[2], P. C. Burda[3], S. Tenzer[2], T. W. Gilberger[3], G. Pradel[1]

[1]RWTH Aachen University, Cellular and Applied Infection Biology, Aachen, Germany
[2]Johannes-Gutenberg University Mainz, Institute of Immunology, Mainz, Germany
[3]Bernhard Nocht Institute, Centre for Structural Systems Biology, Hamburg, Germany

Introduction

Malaria tropica, caused by protozoan parasite *Plasmodium falciparum* remains an important health threat. Molecular understanding of *P. falciparum* gametocytes and their egress from the host cell is crucial for the development of transmission-blocking strategies, which are needed for malaria eradication and antimalarial resistance control. Following uptake by the mosquito vector, gametocytes become activated through environmental stimuli, which triggers their inside-out egress from the red blood cell. At least two types of specialized secretory vesicles are essential for this process. Initially, the parasitophorous vacuole membrane ruptures after exocytosis of osmiophilic bodies (OB) followed by lysis of the erythrocyte membrane, which is associated with exocytosis of g-exonemes. Known components of OBs are G377 and MDV1, while for g-exonemes only PPLP2 has as yet been identified as a constituent.

Material and Methods

To unveil the proteomes of OBs and g-exonemes, we employed BioID methods. BioID/TurboID lines for G377, MDV1 and PPLP2 were generated to identify possible interaction partners and biotinylated proteins of all three lines were successfully analysed via mass spectrometry (MS).

Results

For selected putative vesicle components as identified by MS, HA-tagged parasite lines were generated using the pSLI-HA-*glmS* knockdown system. Among others, two unknown conserved proteins, PF3D7_1319900 and PF3D7_0811600*,* and the secreted ookinete protein PSOP1 were shown to localized to vesicular structures in gametocytes. The lines are currently being phenotypically characterized by means of immunohistochemical techniques and cell-based assays to investigate their role in egress-related vesicle dynamics.

Conclusion

The results help to unveil the interactome of egress-related vesicles of *P. falciparum* gametocytes, providing insights into the immense molecular machinery involved in mediating egress of malaria parasites from erythrocytes during gametogenesis.

P12

Hold tight: Trans-sialidase allows *Trypanosoma congolense* to attach to host cells

J. Rosenau[1], J. Lisack[2], J. Bargul[2], M. Engstler[2]

[1]Universität Bremen, Bremen, Germany
[2]Universität Würzburg, Würzburg, Germany

Nagana, an animal disease caused by African trypanosomes, affects both wild and domestic animals. Cattle are especially susceptible and cause the economic loss of roughly 1 billion US dollars annually, crippling developing countries in sub-Saharan Africa. Among the several trypanosome species which cause nagana, *Trypanosoma (T.) congolense* is the most pathogenic. A key feature involved in *T. congolense* infection in both the mammalian host and the insect vector, the tsetse fly, is its ability to attach to host cells such as red blood cells (RBCs), blood vessel walls, and the fly proboscis. This attachment can harm host cells and is done in a sialic acid dependent manner. While the mechanism of this attachment has been hypothesized to be proteins of the trans-sialidase family, this has never been proven. Here, we aimed to better characterize this attachment and its mechanism in *T. congolense*. We first performed binding assays by adding RBCs from different host species to *T. congolense* culture. We found that *T. conoglense* preferentially binds to host species which have alpha 2-3 linked sialic acid on their RBC surface. We then pre-treated either *T. congolense* cells or host RBCs with sialidase or with different sialic acid donors and acceptors and checked for binding. These results strongly point to *trans*-sialidase being the cause of binding. We then verified these results *in vitro* by immobilizing recombinant trans-sialidase (TconTS) and repeating binding experiments with RBCs from different host species as well as pre-treatment with sialic acid donor or acceptor substrates. Finally, we aimed to introduce TconTS into a sister species of trypanosome, *Trypanosoma brucei,* which does not attach to host cells, to show that TconTS is truly the cause of binding. Taken together, these results show that *T. conoglense* preferentially binds to certain host species, that *trans*-sialidase is the cause of parasite binding to host tissues, and, as binding is crucial to parasite survival in both the mammalian host and insect vector, is a potential target against AAT.

P13

Insights into the molecular IgE-IPSE/alpha-1 interaction responsible for basophil activation

P. F. M. Naujack[1], F. H. Falcone[1], T. S. Jardetzky[2]

[1]Institute for Parasitology (Parasitologie/Zoonosen), BFS, JLU Gießen, Gießen, Germany
[2]Institute for Structural Biology, Stanford University, Stanford, CA, United States

IPSE/alpha-1 (IL-4 inducing principle of *S. mansoni* eggs) is a glycoprotein secreted by the eggs of the blood trematode *S. mansoni*, the causative agent of schistosomiasis, an important neglected tropical disease. Natural IPSE occurs as a dimer, in which an unpaired cysteine (C132) is responsible for homodimerization. IPSE has been shown to bind to IgE, resulting in the release of IL-4 and IL-13 from basophils and mast cells. The classical mechanism of IgE-dependent activation consists of cross-linking IgE by allergen binding to the antigen-recognition variable region of the corresponding immunoglobulin. While IPSE must occur as homodimer for successful IgE binding, this protein appears to activate basophils by binding to IgE without any typical cross-linking.

The aim of this study is to investigate the molecular details underlying this unique interaction between IPSE/alpha-1 and IgE.

Using site-directed mutagenesis, we created several mutants, based on the knowledge that neither IPSE monomers nor the T92Y/R127L mutant, are able to activate basophils. Proteins were expressed in HEK293-6E suspension cells, followed by affinity chromatography for purification. The ability of all IPSE forms to activate basophils by binding IgE was evaluated using humanized RS-ATL8 rat basophilic leukemia (RBL) reporter cells. Cells were sensitized with either IgE-containing sera or different IgE truncates and luciferase expression was measured after stimulation with IPSE. Ancillary ELISAs using similar truncated forms of IgE and WT IPSE were performed in order to further determine the binding region.

Our results show that all the mutations have an impact on IPSE"s capability to interact with IgE, thus lowering the activation of the reporter cells. Only the double mutant T92Y/R127L hampers cell activation completely, leading us to the conclusion that both amino acids must be key residues involved in IgE interaction. Another reason could be that these mutations cause a conformational change of the protein, resulting in incorrect binding to IgE. Furthermore, we show that WT IPSE does not bind to all truncated forms of IgE, suggesting that IPSE needs all heavy chain domains, with or without light chains, to successfully bind to IgE.

The proper folding of the IPSE mutants will be validated by thermal shift assay. Ultimately, Negative Staining of the ternary IgE/IPSE/FcεRIα complex are expected to reveal a detailed model of interaction.

P14

Probing into the molecular pathway leading to *Plasmodium falciparum* egress from the red blood cell

K. Niedermüller[1,2], V. Lochte[1,2], J. S. Wichers[1,2], T. W. Gilberger[1,2], P. C. Burda[1,2]

[1]BNITM, Molekulare Parasitologie, Hamburg, Germany
[2]Centre for Structural Systems Biology, Molekulare Parasitologie, Hamburg, Germany

Introduction: During their development within the vertebrate host, *Plasmodium* parasites invade and proliferate in hepatocytes and red blood cells. Within these cells, parasites are surrounded by a parasitophorous vacuole membrane (PVM). Consequently, for their release from host cells and to propagate the infection, parasites have to disrupt the PVM and the host cell membrane in the process of egress. Despite its importance, only a few parasite proteins have been identified so far to be involved in host cell egress and the underlying molecular mechanism is not well understood. An important regulator of egress is the subtilisin-like serine protease 1 (SUB1), which localizes to specialized secretory organelles. SUB1 is released into the parasitophorous vacuole, where it activates other proteins involved in egress.

Objectives: In this study, we aimed to identify novel proteins that might be processed by SUB1 and that might exert important functions for parasite release from host cells.

Materials & methods: To probe into the pool of SUB1 substrates, we generated parasites conditionally expressing SUB1 fused to the ascorbate peroxidase APEX2 and used these for proximity-dependent biotinylation.

Results: Mass spectrometry-based analysis of SUB1-APEX2 parasites after proximity-dependent biotinylation resulted in 24 putative SUB1 interactors, including 13 known or predicted SUB1 substrates. To understand their function, especially in light of parasite egress from its host cell, we are currently characterizing four of these potential SUB1 interactors using reverse genetics.

Conclusion: Parasites conditionally expressing SUB1 fused to the ascorbate peroxidase APEX2 might be a powerful tool to screen for novel SUB1 substrates.

P15

An experimental tool to study exportin-1-dependent nuclear export in *Plasmodium falciparum*

L. Plutowski[1], A. Kudulyte[1], J. Niethammer[1], K. Grundhöfer[1], S. Klaus[1], M. Ganter[1]

[1]Centre for Infectious Diseases, Parasitology, Heidelberg, Germany

Severe human malaria is caused by *Plasmodium falciparum*. In the clinically relevant blood stage of infection, *P. falciparum* proliferates through an unusual cell cycle mode called schizogony. During schizogony, the parasite"s nuclei multiply several times and a multinucleated cell is formed before daughter merozoites emerge. To facilitate rapid parasite proliferation, the nuclei multiply asynchronously despite residing in a shared cytoplasm. Moreover, the DNA replication fork protein *P. falciparum* PCNA1 accumulates only in those nuclei that replicate their DNA. One possibility to achieve this heterogenous nuclear accumulation is regulated nucleocytoplasmic transport. Especially regulated nuclear export may allow for protein accumulation in individual nuclei as nuclear export receptors bind their cargo in the confinements of the nucleus. In human cells, the nuclear export receptor exportin-1 transports roughly 1000 cargo proteins and has also been implicated in the export of the human PCNA1 homologue. Exportin-1-dependent nuclear export can be studied with the inhibitor leptomycin B (LMB), which covalently binds to a cysteine residue in the cargo binding pocked of exportin-1. However, *P. falciparum* is refractory to LMB inhibition as this cysteine is not conserved and a potential role for exportin-1 in the nuclear export of PCNA1 remains elusive. To develop an experimental tool, we genetically engineered *P. falciparum* by mutating isoleucine 637 of exportin-1 to cysteine (I637C). This rendered *P. falciparum* sensitive to LMB (EC_{50} ~185 nM). Currently, we are characterizing the I637C mutant and exportin-1-dependent nuclear export using synthetic and predicted canonical cargo proteins. In parallel, we fused predicted nuclear import and export signals of *P. falciparum* PCNA1 to GFP. The localization of these constructs suggests that *P. falciparum* PCNA1 harbors at least one functional nuclear export signal. Together, this work will provide an experimental tool to study exportin-1-dependent nuclear export in *P. falciparum*. Investigating the nucleocytoplasmic transport will also reveal its relevance for heterogeneous nuclear accumulation of *P. falciparum* PCNA1. A better understanding of the fundamental biology of *P. falciparum* may also pave the way for new intervention strategies to curb malaria.

P17

The histone methyltransferase DOT1B is dispensable for stage differentiation in *Leishmania mexicana* but not for efficient infection of macrophages

E. T. Rauh[1], N. Eisenhuth[1], F. Butter[2], A. Debus[3], U. Schleicher[3], C. J. Janzen[1]

[1]Cell- and Developmental Biology, Zoologie I, Würzburg, Germany
[2]Institute of Molecular Biology, Mainz, Germany
[3]University Hospital Erlangen, Microbiology-Institute Clinical Microbiology, Erlangen, Germany

1 Introduction

One of the greatest challenges for eukaryotic cells is to organise and maintain a dynamic genome architecture. This allows precise temporal and spatial access to the chromatin fibres to regulate nuclear processes such as transcription, DNA repair and replication. Post-translational histone modifications such as methylation of lysine or arginine residues are one device in the nuclear tool box to facilitate required changes of chromatin structure. DOT1 (disruptor of telomeric silencing 1) is a conserved histone methyltransferase that mediates methylation of lysine 79 of histone H3 (H3K79me) in many eukaryotes. In trypanosomatids, two homologues of DOT1, DOT1A and DOT1B, have been identified and are best characterised in *Trypanosoma brucei*. While *Tb*DOT1A mediates mono- and di-methylation of H3K76, *Tb*DOT1B can additionally catalyse tri-methylation of this residue (H3K76me3). *Tb*DOT1A is necessary for replication initiation and thus essential for the survival of the cells. *Tb*DOT1B is not essential for parasite growth in cell culture but is required for efficient *in situ* switching of variant surface glycoproteins. Interestingly, *Tb*DOT1B-depleted bloodstream forms are not able to differentiate to procyclic forms. Substantially less is known about the functions of DOT1 enzymes in *T. cruzi* and to date nothing is known about their role in *Leishmania* parasites.

2 Objectives

Here, we aimed to elucidate whether the functions of DOT1 histone methyltransferases are conserved in *Leishmania mexicana.*

3 Results

As in *T. brucei*, the H3K76 methylation pattern is also cell cycle-regulated in *L. mexicana* with H3K76me1/2 only detectable in G2 phases and mitosis of the cell cycle. As in *T. brucei*, we observed that *Lmx*DOT1B is not essential for growth of procyclic promastigotes *in vitro*. CRISPR Cas9-mediated *Lmx*DOT1B deletion resulted in a loss of H3K76me3, indicating that *Lmx*DOT1B is solely responsible for this histone modification. Strikingly, and in contrast to *T. brucei*, *Lmx*DOT1B is not essential for differentiation of axenic procyclic promastigotes to amastigotes *in vitro*, but important to establish an infection of bone marrow-derived macrophages.

4 Conclusion

These data suggest that although the enzymatic activity of *Lmx*DOT1 enzymes is conserved, *L. mexicana* parasites have developed novel functions specific to their life cycle.

P18

Bsep/*Abcb11* knockout ameliorates *Schistosoma mansoni* liver pathology by reducing parasite fecundity

M. Sombetzki[1], T. Macháček[2], C. D. Fuchs[3], F. Winkelmann[1], M. Trauner[3]

[1]Rostock University Medical Center, Department of Tropical Medicine and Infectious Diseases, Rostock, Germany
[2]Faculty of Science, Charles University, Department of Parasitology, Prague, Czech Republic
[3]Medical University of Vienna, Vienna, Austria

Background & Objectives: *Schistosoma mansoni* infection is one of the worldwide leading causes of liver fibrosis and portal hypertension. The objective of this study was to evaluate whether polyhydroxylated bile acids, known to protect mice from development of acquired cholestatic liver injury, counteract *Schistosoma mansoni*induced inflammation and fibrosis.

Materials & Methods: Adult FVB/N WT and *Abcb11*/*Bsep* KO mice were infected with either 25 or 50 *Schistosoma mansoni* cercariae. Eight weeks post infection, effects on liver histology, serum biochemistry, gene expression profile of pro-inflammatory cytokines and fibrotic markers, hepatic hydroxyproline content as well as FACS analysis were performed.

Results: *Bsep* KO mice infected with *Schistosoma mansoni* showed significantly less hepatic inflammation and tendentially less fibrosis compared to infected WT mice. Despite elevated ALT, AST, and AP levels in infected *Bsep* KO mice, inflammatory cells such as M2 macrophages and Mac-2/galectin-3+ cells were reduced in these animals. Accordingly, mRNA-expression levels of anti-inflammatory cytokines (IL-4; IL-13) were increased in *Bsep* KO mice upon infection. Furthermore, infected *Bsep* KO mice exhibited decreased hepatic egg load and parasite fecundity, consequently affecting the worm reproduction rate. Mechanistically these findings may at least in part be attributed to elevated serum bile acid levels and consecutively lower blood pH in infected *Bsep* KO mice.

Conclusions: The loss of *Bsep* and the resulting changes in bile acid composition and blood pH reduce parasite fecundity, thus attenuating development of *S. mansoni* induced hepatic inflammation and fibrosis.

P19

Identification of parasite-associated apoptosis signaling pathways in neurocysticercosis

L. E. Sternkopf[1], F. U. Prodjinotho[1], K. Schumann[1], C. Sikasunge[2], J. Esser-von Bieren[3], C. Prazeres da costa[1]

[1]Institute for Medical Microbiology, Immunology and Hygiene, TUM, Munich, Germany
[2]University of Zambia (UNZA), School of Veterinary Medicine, Department of Paraclinical Studies, Lusaka, Zambia
[3]Helmholtz Center Munich, Center of Allergy and Environment (ZAUM), Munich, Germany

Introduction:

In human neurocysticercosis (NCC), caused by brain cysts of the pork tapeworm *T. solium*, and currently the most common cause of childhood epilepsy in many endemic areas worldwide, the immunopathogenesis and disease severity are determined by the viability of the cysts. Whereas viable cysts in the brain are associated with asymptomatic disease, decaying cysts, following treatment can cause severe inflammation, headaches and epileptic seizures. We recently showed that decaying cyst material causes brain microglia activation and peripheral immune cell apoptosis by yet unidentified mechanisms.

Objectives: Within this project, we aim to investigate the proapoptotic potential of decaying cyst (cyst vesicular fluid =CVF) on immune cell populations (T cells, monocytes, macrophages and microglia), the inflammatory mediators and pathways and associated CVF active molecules.

Materials and methods: Isolated human peripheral blood immune cells, monocytes as well as murine bone marrow derived macrophages and microglia were treated with CVF of cysts collected from naturally infected pigs. Cellular apoptosis and necrosis were analyzed using flow cytometry and culture supernatants were then screened for different inflammatory mediators (e.g. TNFα, ROS, Fas). Apoptosis pathways were identified following FLICA active caspases, selective ligand receptors blocking assays and CVF inducing molecules characterized the mean of mass spectrometry and biological function screening.

Results: CVF induced barely necrosis but rather apoptosis in a concentration and time dependent manners and targeting preferentially monocytes and CD3- cell populations more than CD3+ T cells. CVF molecules, potentially cathepsin B/D and calpains, promoted significant TNF-α from peripheral cells and TGF-ß from microglia as potential drivers of cellular apoptosis pathways and epileptic development.

Conclusion: With this work, we uncovered determinant mechanisms in cellular apoptosis underlying the immunopathogenesis of NCC that may offer an important therapeutic strategy in inflammatory epilepsy and NCC treated patients.

P20

Occurrence of *Perkinsus olseni* and other parasites in New Zealand Greenshell™ mussels (*Perna canaliculus*)

F. Muznebin[1,2], *A. C. Alfaro*[1], *S. C. Webb*[3]

[1]Auckland University of Technology, Aquaculture Biotechnology Research Group, School of Science, Auckland, New Zealand
[2]Jagannath University, Department of Zoology, Faculty of Life and Earth Science, Dhaka, Bangladesh
[3]Cawthron Institute, Nelson, New Zealand

The New Zealand Greenshell™ mussel (*Perna canaliculus*) is endemic to New Zealand and is the top shellfish export product. However, the growth of this industry is being adversely affected by potential disease outbreaks. To assess these health threats, a detailed histopathological examination of a targeted survey/sampling of farmed mussels (*P. canaliculus*) was undertaken. Histological sections followed by confirmatory *in situ* hybridization (ISH) resulted in the identification of *Perkinsus olseni* (56% prevalence). Apicomplexan parasite X (APX) (78%), copepods (*Pseudomyicola spinosus* or *Lichomolgus uncus*) (1%), *Microsporidium rapuae* (1%), intracellular microcolonies of bacteria (IMCs) (2%) and, bacilli and cocci bacteria (4%) were also identified across organs. There was a significant association between *P. olseni* and APX infection in mussels. The abundance of *P. olseni* and APX in *P. canaliculus* was evaluated semi-quantitatively using two separate modified grading scales, which are suitable to assess *P. olseni* and APX infection of the histological section. Immunological tissue responses (haemocytosis and ceroid material) were also evaluated semi-quantitatively and were significantly associated with *P. olseni* and APX. The pathologies of digestive tubules and gills were observed in this study and *P. olseni* infection is related to digestive tubule deterioration (large lumen, with a thin epithelial wall). A significant association between the presence of parasites and the health conditions (healthy and unhealthy) of mussels were observed. Therefore, the findings in this study provide information regarding the infections of potential parasites and health conditions in New Zealand Greenshell™ mussels (*P. canaliculus*).

Keywords: Apicomplexan X; Ceroid material; Haemocytosis; *In situ*–hybridization; *Perkinsus olseni*; Histology.

P21

Characterization of novel *Plasmodium berghei* liver stage-specific CD8$^+$ T cell epitopes

T. Song[1], K. Matuschewski[1]

[1]Humboldt-Universität zu Berlin, Molecular Parasitology , Berlin, Germany

Introduction

Plasmodium berghei generates a complex immune response when infecting its rodent host. Pathogenesis of the infection is not yet fully elucidated, but from our best understanding, IFNγ$^+$CD8$^+$ T cells are considered crucial for the protection during pre-erythrocytic stage infection. T cell based-vaccines targeting different stages of the *Plasmodium* life cycle are under development. The prime sporozoite antigen for recombinant vaccines development is the circumsporozoite protein (CSP), but recent studies suggest that the immune responses towards CSP are dispensable for sterile protection in murine models. This highlights the importance of identifying other candidate antigens and epitopes.

Objectives

To profile liver stage-specific CD8$^+$ T cell epitopes that contribute to the immune protection against *P. berghei* infection.

Materials & methods

In this project, we carried out *in silico* epitope prediction, as well as *in vivo* and *ex vivo* immunological studies in murine models to characterize CD8$^+$ T cell epitopes that elicit protective immune responses against *P. berghei* liver-stage infection.

Results

We detected several new CD8$^+$ T cell epitopes that stimulate IFN-γ secretion in antigen-experienced cells for protection. In addition to established *P. berghei* liver-stage antigens, such as TRAP, S20 and GAP50, novel antigens were also identified.

Conclusion

The discovery of novel *P. berghei* liver stage-specific CD8$^+$ T cell epitopes provides new candidates for malaria vaccine design and expands our understandings on the immune correlates of protection against *Plasmodium*infection.

P22

The genetically encoded calcium indicator GCaMP3 reveals spontaneous calcium oscillations at asexual stages of the *Plasmodium falciparum* parasite

B. M. Dos Santos[1], M. Fila Pecenin[1], L. Borges-Pereira[1], D. C. Martins-Jr[2], R. F. Hashimoto[3], C. R. d. S. Garcia[1]

[1]University of São Paulo, Department of Clinical and Toxicological Analysis, São Paulo - SP, Brazil
[2]Federal University of ABC, Center of Mathematics, Computing and Cognition, Santo André, Brazil
[3]University of São Paulo, Institute of Mathematics and Statistics, São Paulo - SP, Brazil

Introduction - Calcium ions (Ca^{2+}) are versatile intracellular messengers capable of regulating different cellular functions. Oscillations in cytosolic Ca^{2+} concentrations activate essential mechanisms for the survival of *Plasmodium falciparum*. **Objectives -** The protocols used to study the dynamics of Ca^{2+} in the malaria parasite are based on the dyes, which are invasive and do not allow discrimination between the signal from the host cell and the parasite. We have developed the PfGCaMP3 parasites, an innovative strain and tool for studying spontaneous intracellular Ca^{2+} oscillations without external markers. **Materials & methods -** Using the PfGCaMP3 strain, visualized on a Nikon Ti2-E real-time fluorescence microscope, we demonstrated the occurrence of spontaneous Ca^{2+} oscillations in the human malaria-causing parasite *Plasmodium falciparum* during intraerythrocytic phases: ring, trophozoite and schizont. **Results -** To verify the changes in the fluorescence of the GCaMP3 construct, indicative of cytosolic Ca^{2+} fluctuations, we used the Fourier transform, which was applied to the fluorescence intensity data extracted from different experiments. Thus, it was possible to verify that spontaneous cytosolic Ca^{2+} oscillations occur in the three intraerythrocytic stages of the parasite, with most oscillations occurring in the ring and trophozoite stages. **Conclusion -** This tool is promising for studying pharmacological compounds that may interfere with calcium dynamics in *P. falciparum*.

P23

Characterizing the pathological events of human lung endothelial cells during *P. falciparum* infection

H. Torabi[1], Y. Wu[1], M. D. P. Martinez Tauler[1], B. Honecker[1], K. Höhn[1], I. Bruchhaus[1], N. Galal Metwally[1]

[1]BNITM, RG Host Parasite Interaction, Hamburg, Germany

Characterizing the pathological events of human lung endothelial cells during *P. falciparum* infection

Hanifeh Torabi, Yifan Wu, Maria del Pilar Martinez Tauler, Barbara Honecker, Katharina Höhn, Iris Bruchhaus, Nahla Galal Metwally

Bernhard Nocht Institute for Tropical Medicine, RG Host Parasite Interaction, Hamburg, Germany

Introduction

Plasmodium falciparum is the main cause of morbidity and mortality in malaria patients, especially in children under five years of age. Understanding the pathogenesis of *P. falciparum* infection in the context of host-parasite interaction could lead to targets for adjunctive therapies. The sequestration of *P. falciparum*-infected erythrocytes (*Pf*IEs) in the vascular bed of various organs, such as the brain, heart, lung, stomach, and kidney, allows the parasite to escape elimination by the spleen. Sequestration occurs due to an interaction between a parasite ligand presented on the surface of *Pf*IEs and a number of human endothelial cell receptors (ECRs). This cytoadhesion is mainly attributed to members of the *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) family, encoded by approximately 60 *var* genes per haploid parasite genome.

Objectives

Due to a bidirectional interaction between *Pf*IEs and host ECRs, malaria infection can result in severe complications. These interactions likely vary from one tissue to another because of the differences in the ECR expression profiles. We hypothesize that these interactions do take place in the early phases of infection to alter the host's response once the parasite enters the bloodstream in order to survive. In addition, host cells interact with the *Pf*IEs and most likely modify their biology. Some studies suggest that cytoadhesion does not provide the full explanation for the complications associated with malaria. In this context, extracellular vesicles (EVs) as cargo from cell to cell, carrying proteins and nucleic acids, are being investigated to gain a better understanding of the interaction between parasite and host. This study aimed to investigate the transcriptomes of lung ECs exposed to different stimuli occurring during *P. falciparum* infection in order to analyze the different profiles of tissue-specific *Pf*IEs communication.

Results

We were able to enrich a parasite population that specifically binds to lung ECs. We are currently analyzing the transcriptomes of this parasite population. Furthermore, the miRNA profile of EVs secreted by lung ECs during *P. falciparum* infection is being determined.

P24

Increased HIV incidence in *Wuchereria bancrofti* microfilaria positive individuals in Tanzania

J. Mnkai[1], M. Ritter[2], J. Minich[3], M. Chachage[1,4], W. Olomi[1], L. Maganga[1], L. Maboko[5], E. Lelo[6], D. Kariuki[7], A. Y. Debrah[8], S. Horn[9], C. Geldmacher[9,2], E. Saathoff[9,2], M. Hoelscher[9,2], K. Pfarr[10,3], A. Hoerauf[10,3,11], I. Kroidl[9,2]

[1]National Institute of Medical Research, Mbeya Medical Research Center, Mbeya, Tanzania, United Republic Of
[2]German Center for Infection Research (DZIF), Munich, Germany
[3]University Hospital Bonn, Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany
[4]University of Dar es Salaam, Mbeya College of Health and Allied Sciences, Mbeya, Tanzania, United Republic Of
[5]Tanzanian Commision for AIDS, Dodoma, Tanzania, United Republic Of
[6]Kenya Medical Research Institute, Nairobi, Kenya
[7]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
[8]Kwame Nkrumah University of Science and Technology, Kumasi Centre for Collaborative Research, Kumasi, Ghana
[9]Ludwig-Maximilians-Universität (LMU) , Division of Infectious Diseases and Tropical Medicine, Munich, Germany
[10]German Center for Infection Research (DZIF), Bonn, Germany
[11]German-West African Centre for Global Health and Pandemic Prevention (G-WAC), Bonn, Germany

Background: Globally, *Wuchereria bancrofti* accounts for 90% of all lymphatic filariasis cases and is associated with reduced immunity against concomitant infections. Indeed, our previous study described a 2.3 fold increased HIV incidence among individuals with *W. bancrofti* infection, as measured by the circulating filarial antigen (CFA) of the adult worm. However, the previous study did not measure microfilariae (MF) in the blood of infected individuals, a factor, which is significant because adaptive immune responses in filarial infected individuals differ according to MF status. This new study aimed to retrospectively determine microfilariae status of the participants to assess if the previously described increased HIV susceptibility is associated with the presence of MF in the same cohort.

Methods: CFA positive HIV negative biobanked human blood samples (n=350) were analyzed for *W. bancrofti* MF chitinase by real time PCR.

Results: The PCR provided a positive signal in 12/350 (3.4%) samples. During four years of follow-up (1109 person years (PY)), 22 study participants acquired an HIV infection. In 39 PY of *W. bancrofti* MF chitinase positive individuals, three new HIV infections occurred (7.8 cases per 100 PY) in contrast to 19 seroconversions in 1,070 PY of *W. bancrofti* MF chitinase negative individuals (1.8 cases per 100 PY, p=0.014).

Conclusion: In the subgroup of MF-producing *W. bancrofti*-infected individuals, the HIV incidence exceeded the previously described moderate increased risk for HIV seen in all filarial infected individuals (regardless of MF status). Compared with *W. bancrofti*-uninfected persons from the same area the risk to acquire HIV is approximately 10 times higher. compared. If both *W. bancrofti*-infected subgroups are compared,l the infection with measurable MF is associated with a 4.58 times higher HIV incidence than the W. bancrofti infection without MF in the blood. This study highlights the need to eliminate lymphatic filariasis, not only because of the disabling pathological changes, which can occur, but also because of the impact on susceptibility to HIV and other viruses. Further studies should decipher the mechanisms that are responsible for the increased risk of HIV in microfilaria positive individuals and whether anti-filarial treatment in LF endemic areas can reduce the HIV incidence in filarial infected people.

P25

Antihemostatic factors secreted by parasitic fluke *Schistosoma mansoni*

L. Jedlickova[1], K. Peterková[1,2], J. Dvořák[1,3]

[1]Czech University of Life Sciences, Centre of Infectious Animal Diseases, Prague, Czech Republic
[2]Charles University Prague, Department of Parasitology, Prague, Czech Republic
[3]Czech Academy of Sciences, Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic

Introduction: Hemostasis is a defense mechanism that prevents blood loss by sticking platelets together and forming a fibrin clot at the site of injury. Proteolytic enzymes involved in the hemostatic cascades belong to the group of serine peptidases and their activity must be strictly controlled by endogenous inhibitors. The blood flukes *Schistosoma mansoni*, same as other blood-feeding parasites, have to overcome the hemostasis of host blood and mimic the host regulation mechanism. Thus, they have developed molecular equipment containing a range of anti-hemostatic, anti-inflammatory, and immunomodulatory molecules that contribute to the suppression of the host protective mechanisms and enable parasite survival. In our project, we focus on the life stages of adults and eggs, which both occur in the mesenteric veins and closely interact with host blood elements.

Objectives: The main goal of the project is to identify and characterize bioactive molecules secreted by parasitic flukes and their eggs for the modulation of host hemostasis and to evaluate their potential applications in biomedicine.

Materials & methods: Based on an analysis of transcriptomic and proteomic data of *S. mansoni* adults and mature/ immature eggs, we selected suitable candidate molecules such as annexins, Kunitz-type inhibitors, and serpins for our experiments. Production of their recombinant forms in *E. coli* bacterial system is now in process.

Results: Our preliminary results indicate a significantly different composition of the excretory-secretory products from adults and mature/ immature eggs of *S. mansoni*. Based on the results combined with the transcriptome analysis, we selected several potential antihemostatic candidates.

Conclusion: This project will reveal the functions of selected molecules and identify their role within the complex interaction mechanisms.

P28

Exploring the *Fasciola hepatica* kinome for the discovery of novel drug targets and drug candidates

S. Ajmera[1], A. Stroehlein[2], O. Puckelwaldt[1], B. Pereira-Moreira[1], F. H. Falcone[1], S. Häberlein[1]

[1]Justus-Liebig-Universität Gießen, Institut für Parasitologie, Gießen, Germany
[2]The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

Introduction: Fasciolosis is a globally prevalent zoonosis and a neglected tropical disease caused by infection with liver flukes such as *Fasciola hepatica*. Due to resistance against the main drug in use, triclabendazole, new drug candidates are urgently needed. Protein kinases (PKs) might serve as starting point for the development of new drugs, because they regulate a vast variety of cellular processes including cell growth, proliferation, differentiation and metabolism.

Objective: We pursue the hypothesis that inhibition of PKs may represent a promising therapeutic strategy against fasciolosis. The aim of this project is to identify druggable kinases in *F. hepatica* and potent PK inhibitors.

Material & Methods: To generate a draft kinome dataset of *F. hepatica* we employ a genomic-bioinformatic approach using the programs Kinnanote, OrthoMCL, Exonerate, pBLAT and InterProScan to generate a kinome dataset of *F.hepatica.* This dataset will allow the prioritization of potential target kinases followed by virtual screening of compound libraries against homology models of these prioritized kinases. The top hit compounds will be screened against different life stages of *F. hepatica in vitro*, and their inhibitory activity confirmed against recombinantly expressed PKs. Genetic target validation will be achieved by RNA interference (RNAi) against selected kinases.

Results: A first *F. hepatica* draft kinome contains 225 PKs to form 9 (sub) families. Single-cell transcriptomics data, show that some prioritized PKs are predominately expressed in cells and tissues of therapeutic interest, such as neoblasts, the tegument and neuronal cells. PK inhibitor treatment had lethal activity against both, immature and adult worms 24 hours after treatment at concentrations comparable to triclabendazole.

Conclusion: Preliminary data suggest that targeting PKs may represent an effective approach to control *F. hepatica*. Further investigations will assess the re-purposing potential of PK inhibitors against this parasitic flatworm.

P29

Preliminary studies on the *in vitro* efficacy of the antibiotic nitroxoline in *Naegleria lovaniensis*

K. Jochims[1], *F. Fuchs*[1], *P. Scheid*[1]

[1]Bundeswehr Central Hospital Koblenz, Department of Microbiology and Hospital Hygiene, Koblenz, Germany

1. **Introduction**

Species of the genus *Naegleria* belong to the free-living amoebae that are ubiquitously distributed in the environment. *Naegleria fowleri* is a pathogenic species of this genus, which can cause fatal amoebic meningoencephalitis in humans. Therefore, progress in developing effective therapies against such pathogenic free-living amoebae is of great importance, however only few novel drugs are being developed. We report the efficacy of the old antimicrobial nitroxoline on the non-pathogenic species *Naegleria lovaniensis*, which is closely related to *N. fowleri*.

2. **Objectives**

To investigate the activity of the old and currently repurposed antimicrobial nitroxoline against *N. lovaniensis.*

3. **Materials & methods**

Nitroxoline (Rosen Pharma, St. Ingbert, Germany) diluted in DMSO (MP Biomedicals, Eschwege, Germany) was incubated at different concentrations (range: 0,1 to 32 mg/l) with axenic cultures of *N. lovaniensis* in 24-well-diagnostic plates (Greiner Bio-one, Frickenhausen, Germany). Physical characteristics of cells and cellcounts were constantly assessed with light microscopy including encystation, and hemocytometric determination of living and dead cells within each well. Growth controls were taken from each well to asses the ability of the nitroxoline-exposed amoebaes to regenerate in xenic and axenic cultures. To rule out confounding amebicidal effects of DMSO, each dilution was also investigated with DMSO only, without nitroxoline. *E. coli* ATCC 25922 was used for quality controle of nitroxoline.

4. **Results**

Inhibition and even killing of viable trophozoites and cysts of *N. lovaniensis* was demonstrated at nitroxoline concentrations of 1.0 mg/L - 8.0 mg/L (see Fig. 1)

5. **Conclusion**

Based on the current EUCAST breakpoint for treatment of uncomplicated urinary tract infection (susceptible ≤ 16mg/L for *E. coli*) activity of nitroxoline against *N. lovaniensis* can be considered excellent. Nitroxoline should be further investigated as a promising candidate for treatment of *Naegleria*-infections.

Fig. 1

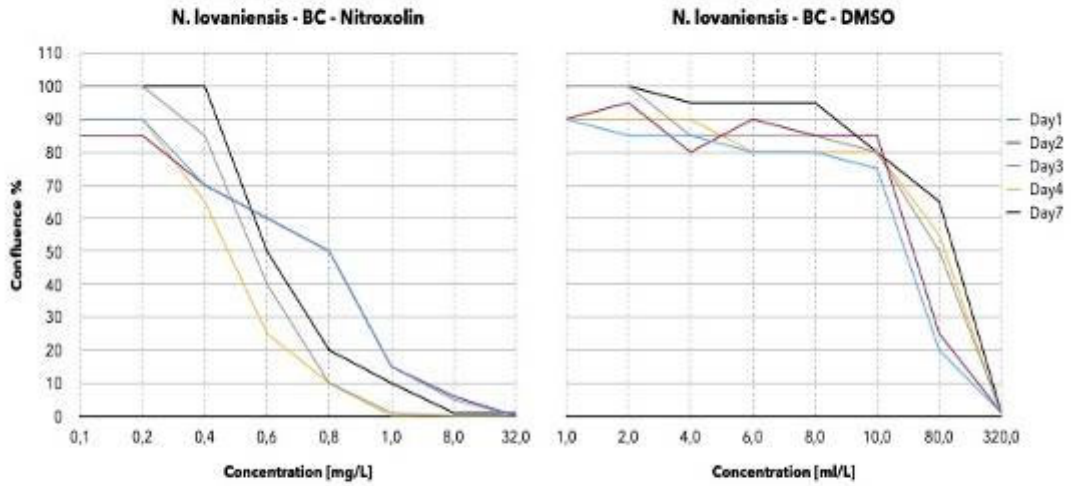


Fig. 1: Confluence in percent of axenic culture of *N. lovaniensis* within the nutrient solution Bacto-Casitone (BC) at Day1, Day2, Day3, Day4 and Day7. **a**: Dilution series with nitroxoline diluted in DMSO; **b**: Dilution series with equally concentrated DMSO without nitroxoline.

P30

In vitro screening of *Schistosoma mansoni* JNK against a prioritized library of predicted type II-kinase inhibitors

B. Pereira-Moreira[1], *S. Grossi Gava*[2], *S. Häberlein*[1], *M. de Moraes Mourao*[2], *F. H. Falcone*[1]

[1]Justus-Liebig-Universität Gießen, Institut für Parasitologie, Gießen, Germany
[2]FIOCRUZ Minas, Belo Horizonte, Brazil

Introduction

Chemotherapy of schistosomiasis relies on a single drug, praziquantel, which efficiently reduces morbidity and mortality due to the disease. The emergence of resistant parasite strains together with the lack of efficiency in killing larval stages transiting through the body substantiate the pursuit for a new drug. Immense efforts have been invested in the discovery of protein kinase (PK) inhibitors; however, given that the majority of PKs are still not targeted by an inhibitor with a useful level of selectivity, there is a compelling need to expand the chemical space available for synthesizing new, potent and selective PKI.

Objective

Small-molecule inhibitors targeting the ATP pocket of the catalytic domain of PKs have potential to become drugs devoid of (major) side effects, particularly if they bind selectively. This is the case for type II PK inhibitors, which cause PKs to adopt the so-called DFG-out conformation, which corresponds to the inactive state of the enzyme. In this conformation, the ligand occupies at the same time the ATP-pocket and an additional space in the cleft between the two lobes of the kinase. Therefore, the goal was to perform a virtual screen against the ATP pocket of S. mansoni JNK in a state where selectivity has more often been observed, to help with potential kinase selectivity issues.

Materials & Methods

Here, we selected the human JNK2 (PDBID: 3NPC) as a template model for the ATP pocket of SmJNK, because it has high sequence identity, great coverage of the protein sequence and was solved with a type II inhibitor. Atomwise performed the screen of a molecular library of several million compounds at the selected target site using AtomNet®, the first deep learning neural network for structure-based drug design and discovery. Its speed and accuracy make it the most advanced technology for small molecule binding affinity prediction.

Results

Top scoring compounds were clustered and filtered to arrive at a final subset of 85 deliverable compounds. These drugs will be screened *in vitro* against larval and adult stages of *S. mansoni*, with additional confocal microscopy analysis of adult worms in order to assess morphological alterations. Moreover, positive hits will be used in our developed fluorescence polarization assay to show the target-ligand interaction.

Conclusion

This study will possibly provide information on novel candidates targeting JNK that can be developed for therapeutic use against schistosomiasis.

P31

Biarylalkyl Carboxylic Acid Amides as Potential Anti-Schistosomal Drugs

S. Reul[1], A. M. Peter Ventura[1], S. Häberlein[2], K. Lange-Grünweller[1], L. Konopka[1], H. Janga[1], W. Obermann[1], A. Grünweller[1], C. G. Grevelding[2], M. Schlitzer[1]

[1]Philips-Universität Marburg, FB16 Pharmazie- Pharmazeutische Chemie, Marburg, Germany
[2]Justus-Liebig-Universität Gießen, Institut für Parasitologie, BFS, Gießen, Germany

Introduction:

Schistosomiasis is a parasitic disease which causes more than 200 000 human deaths per year and about 200 million infections. The Schistosomiasis was declaimed as a neglected tropical disease (NTD) by the WHO. The only therapeutic option that is used against the infection is Praziquantel.

Due to that, the hazard of emerging resistance against Praziquantel is growing. A reduced sensitivity of the parasites against Praziquantel has already been observed. Therefore, the development of new anti-schistosomal agents is essential.

Objectives:

A Novel class of anti-schistososomal agents should be developed. The *in-vitro* activity of the substances should be in 10 mM range. In addition, parasites" physiology and morphology should be impacted, while the compounds should not display cytotoxicity against human cell lines at significantly higher concentrations.

Material & Methods:

Structural variations of several moieties of the biarylalkyl carboxylic acid scaffold were carried out through the synthesis of different derivates of the basic scaffold. Compounds were tested against cultured adult *Schistosoma mansoni* couples and in case of significant activity also against two human cell lines.

Results:

More than 240 compounds with biarylalkyl carboxylic acid moiety were synthesized and tested. The most effective compounds showed anti-schistosomal activity at 10 mM. Furthermore, damaging phenotypes were observed while the agents showed no significant cytotoxicity against human cell lines.

Conclusion:

Using a ligand-based approach to drug design a novel class of anti-schistosomal agents was obtained.

Acknowledgments:

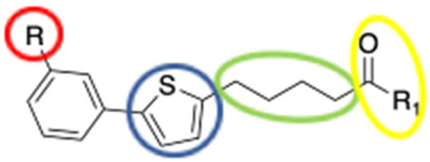
This project was funded by the LOEWE centre DRUID within the Hessian Excellence Initiative and DFG SCHL383-GR1549.

References:

1. S. A. L. Thétiot-Laurent, J. Boissier, A. Robert, B. Meunier, *Angew. Chem. Int. Ed.* **2013**, 52, 7936–7956; *Angew. Chem.* **2013**, 125, 8092–8114.
2. P. G. Fallon, M. J. Doenhoff, *Am. J. Trop. Med. Hyg.* **1994**, 51, 83 – 88.

3. S. S. Botros, J. L. Bennett, *Expert Opin. Drug Discovery* **2007**, *2*, S35 – S40.
4. 4. A. M. Peter Ventura, *et al.*, *ChemMedChem* **2019**, 14, 1856-1862.

Fig. 1



P32

The aldose reductase 1 of *Schistosoma mansoni* – Expression and inhibitor testing for target evaluation

M. E. Überall[1], S. Reul[2], M. Schlitzer[2], P. Czermak[3], D. Salzig[3], C. G. Grevelding[1]

[1]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany
[2]Philips-Universität Marburg, Department of Pharmaceutical Chemistry, Marburg, Germany
[3]Technische Hochschule Mittelhessen - University of Applied Sciences, Institute of Bioprocess Engineering and Pharmaceutical Technology (IBPT), Gießen, Germany

Introduction

Schistosomiasis, caused among others by the parasitic trematode species *Schistosoma mansoni*, leads to chronic inflammation and finally to liver fibrosis. If untreated, the disease can cause life-threatening complications. The current treatment of schistosomiasis is mainly based on a single drug, Praziquantel (PZQ). Due to the frequent use of PZQ, there is upcoming fear of emerging resistance. Therefore, it is necessary to find alternative drugs. Screening of potential drugs is currently based on *in vitro* tests against different stages of the parasite. An attractive alternative is the establishment of enzyme assays with potential target proteins found in the parasite. With the use of such assays, large compound libraries can be tested in high-throughput screenings (HTS) without the need for animal experiments and in a time- and cost-efficient manner.

Objectives

A potential target protein for such HTS, based on its role in detoxification processes in other organisms, is an aldose reductase (AR) orthologue in *S. mansoni* (Smp_053220, *Sm*AR). This assumption is supported by the ubiquitous and sex-independent expression in *S. mansoni* [1, 2] and by a study in *Schistosoma japonicum* where treatment with a specific AR inhibitor showed a clear decrease in worm activity [3].

Materials & Methods

*Sm*AR was recombinantly expressed in *Escherichia coli* strain BL21(DE3) and purified by immobilized metal ion affinity chromatography. The following buffer exchange was carried out performing size exclusion chromatography. Enzyme activity and IC50-values for potential inhibitors were determined using the established enzyme assay.

Results

The enzyme was found to be active *in vitro*. A number of tested compounds, synthesized in the working group of Prof. Schlitzer (Philipps Universität Marburg), showed a clear inhibition of the enzyme and provide a solid basis for optimization and further development.

Conclusion

The successful purification of *Sm*AR in sufficient amounts and the confirmed activity in the enzyme assay enabled first inhibitor screenings. Next steps will be further characterisation of the enzyme itself and possibly later on the performance of HTS.

Literature

[1] Wendt G, et al. Science. 2020;39(6511):1644-1649

[2] Lu Z, et al. Sci Data. 2017;4:170118

[3] Liu J, et al. Parasites & Vectors. 2013;6:162

P33

Schistosomal helicase eIF4A – A potential target of plant-derived rocaglates?

S. Welsch[1], F. Magari[2], S. Häberlein[1], A. Grünweller[2], C. G. Grevelding[1]

[1]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany
[2]Philipps-University Marburg , Institute of Pharmaceutical Chemistry, Marburg, Germany

Introduction:

Schistosomiasis is caused by an infection with blood flukes of the genus *Schistosoma*, with *S. mansoni* being one of several species infectious for humans. No vaccine exists, and treatment of schistosomiasis relies on a single drug - Praziquantel. There is justified fear for the development of resistance, encouraging the search for new targets and drugs.

One potential new drug target is the eukaryotic translation initiation factor 4A (eIF4A), an RNA helicase that unwinds stable RNA secondary structures in the 5'-untranslated region of mRNAs. Once the secondary structures are dissolved, ribosomes can access the mRNA and initiate protein synthesis.

Rocaglates, a compound class isolated from plants of the genus *Aglaia*, prevent ribosome binding and thus protein synthesis by inhibiting eIF4A. Thereby, rocaglates clamp the mRNA on the surface of eIF4A by interacting with a specific phenylalanine (Phe163) of human eIF4A.

Objectives:

We aim to investigate the functions of schistosomal eIF4A and to clarify whether targeting eIF4A of *S. mansoni* by rocaglates could be a therapeutic option in the future.

Material & Methods:

Adult *S. mansoni* worms were treated with rocaglates *in vitro*. Effects of the treatment on stem cell proliferation were analyzed by an EdU assay.

Two schistosomal eIF4A isoforms, one with the specific Phe and one without, were cloned and recombinantly expressed in *Escherichia coli*, purified by affinity chromatography, and used for a thermal shift assay to validate the interaction of rocaglates and schistosomal eIF4A.

Results:

Treatment of adult *S. mansoni* with rocaglates resulted in a reduced vitality and decreased numbers of proliferating EdU-positive cells. First thermal shift assays indicate that only the Phe-containing isoform can be bound by natural and synthetic rocaglates.

Conclusion:

Schistosomal eIF4A may play a role for proliferating cells in adult *S. mansoni.* The interaction of rocaglates and the Phe-containing isoform as well as the vitality effects of these plant-derived products on *S. mansoni* motivate for further evaluation of schistosome helicases as potential targets.

P34

First report of *Orthohalarachne diminuata* (Acari: Halarachnidae) in a captive South American sea lion (*Otaria flavescens*)

D. Ebmer[1,2], E. Kniha[3], V. Strauss[4], A. Kübber-Heiss[4], L. Komornik[2], F. Balfanz[5], S. Hering-Hagenbeck[5], J. Walochnik[3], U. Gärtner[6], H. Prosl[2], A. Taubert[1], T. Voracek[2], C. Hermosilla[1]

[1]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany
[2]Veterinary Clinic Vienna Zoo, Vienna, Austria
[3]Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
[4]Research Institute of Wildlife Ecology, Department for Interdisciplinary Life Sciences, Vetmeduni Vienna, Vienna, Austria
[5]Vienna Zoo, Vienna, Austria
[6]Institute of Anatomy and Cell Biology, Justus Liebig University Giessen, Gießen, Germany

Introduction: Endoparasitic mites of the genera *Halarachne* and *Orthohalarachn*e (Acari: Halarachnidae) parasitize different segments of the respiratory tract of pinnipeds and sea otters. Knowledge on their exact pathogenic potential and on occurrence in pinnipeds in zoological gardens is very scarce.

Materials and methods: A two-year-old South American sea lion (*Otaria flavescens*), born at the Vienna Zoo, died during general anesthesia for pre-transport examinations. Immediately after death, necropsy was performed and tissue samples for histological, bacteriological and parasitological analyses were taken.

Results: At necropsy, 45 adult *Orthohalarachne diminuata* mites were detected in the lower respiratory tract. After trepanation, 410 larval and nymphal specimens were detected in sinus paranasalis. Macroscopically, sinus mucosa showed multiple petechial hemorrhages and histopathological analyses revealed mite cross-sections, sanioserous exudate and epithelial exfoliation. For the first time, *O. diminuata* was molecularly characterized.

Conclusions: Our study constitutes the first record of an *O. diminuata* infestation in a captive *O. flavescens*. We present clinical and pathological data and discuss the etiology of this infestation. Further studies on pathogenic effects of these nasal/nasopulmonary parasites are essentially required for a better understanding of neglected orthohalarchniosis in pinnipeds.

P35

The microclimatic adaptation of *A. cahirinus* throughout Jordan's Great Rift Valley serves as a novel paradigm for natural selection in the animal's eukaryome

E. Al-khlifeh[1]

[1]Al-Balqa' Applied University, Al-Salt, Jordan

Geology and climate factors influence biogeography patterns. Speciations, divergence, physical and biological interactions among species occupying biogeographic zones are all clear evolutionary processes. As a result, it can be used to extract data on the patterns of biological communities and their sympions 'phylosymbiosis,' a term coined primarily for the gut microbial population. The bulk of these sympions is bacteria. Nevertheless, parasites are known to be common inhabitants of animals. Its effects on health, physiology, and the response of its hosts to stress, are becoming more acknowledged. However, it is known how the Eukaryotic population reflects the phylogeny of the animals at the biogeographic level. The rodents used in this investigation *A. cahirinus* and *Mus*. *musculus* were collected from two biogeographic zones: tropical (Sudanese) and temperate (Mediterranean), straddling the Great Rift Valley, ending in Jordan's south. These Bio zones are thought to aid evolutionary and geological developing processes. On the other hand, their topography is distinct (the "Sudanian" faces the African continent from the south, and the "Mediterranean" locates at a slop facing the European continent from the north). The hills are only a few kilometers apart. There is up to ten times more illumination in the Sudanese bio-climatic zone. As a result, it's a great place to learn about phylosymbiosis. Using the mitochondrial D-Loop sequencing, the phylogeny of *A. cahirinus* and *Mus. musculus* was revised. For the Eukaryome identification, the 18S method was employed. Based on this, we evaluate the Eukaryome of animals from different temperature zones across the rift valley. Despite the absence of intra-phylogenetic differences the rodent from the same species. Eukaryome found in the Sudanian zone differ significantly from Mediterranean zones. According to our findings, the gut Eukaryome structure is environmentally adaptive, in response to microclimatic conditions in opposing tropical and temperate biogeographical zones causing subsequent parasite diversification.

Fig. 1

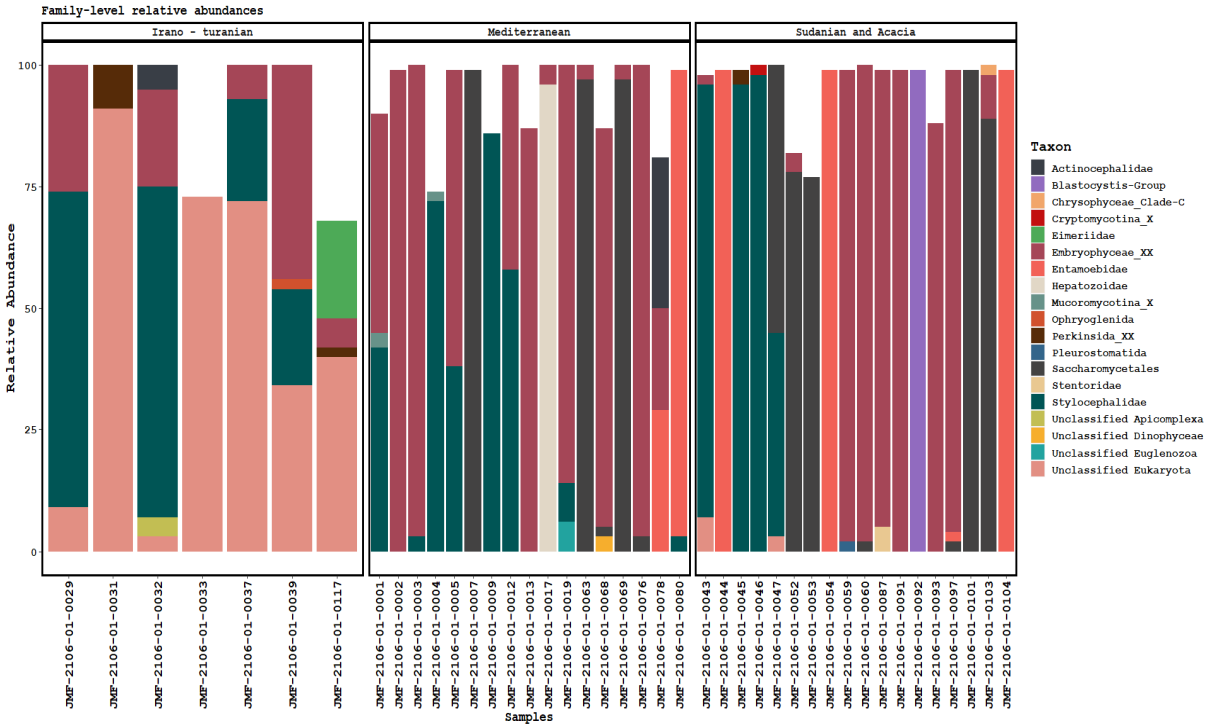
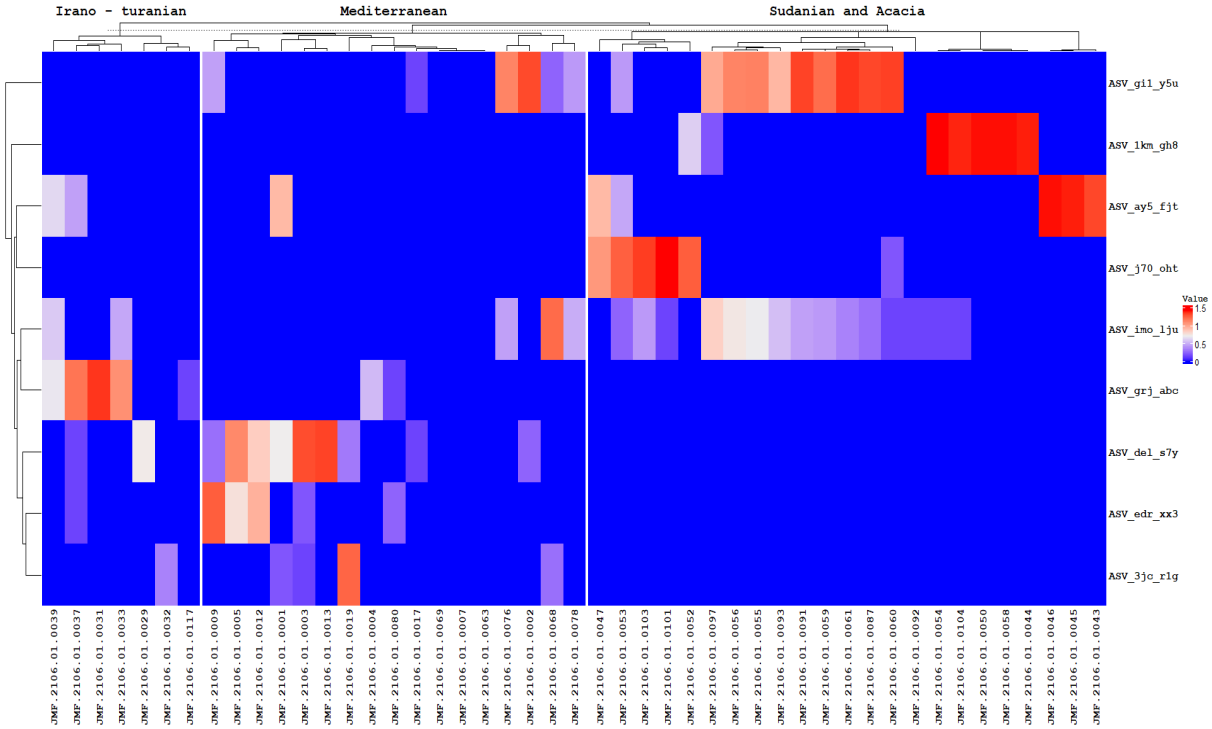

Family-level relative abundances
Irano - turanian
Mediterranean
Sudanian and Acacia
Relative Abundance
100
75
50
25
0
Samples
Taxon
Actinocephalidae
Blastocystis-Group
Chrysophyceae_Clade-C
Cryptomycotina_X
Eimeriidae
Embryophyceae_XX
Entamoebidae
Hepatozoidae
Mucoromycotina_X
Ophryoglenida
Perkinsida_XX
Pleurostomatida
Saccharomycetales
Stentoridae
Stylocephalidae
Unclassified Apicomplexa
Unclassified Dinophyceae
Unclassified Euglenozoa
Unclassified Eukaryota

Fig. 2


Irano - turanian
Mediterranean
Sudanian and Acacia
ASV_gil_y5u
ASV_1km_gh8
ASV_ay5_fjt
ASV_j70_oht
ASV_imo_lju
ASV_grj_abc
ASV_del_s7y
ASV_edr_xx3
ASV_3jc_r1g
Value
1.5
1
0.5
0

P36

Evaluation of between-host heterogeneity in the patterns of immune gene expression in *Hepatocystis* parasite-infected African epauletted fruit bats

B. M. Mugambi[1], D. Reeder[2], I. Ejotre[1,3], K. Field[2], J. Schaer[1]

[1]Humboldt-Universität zu Berlin, Molecular Parasitology , Berlin, Germany
[2]Bucknell University, Lewisburg, PA, United States
[3]Muni University, Arua, Uganda

Hepatocystis parasites are the closest relatives of the mammalian *Plasmodium* species. The genus currently contains up to 25 known species. *Hepatocystis* parasites are known to infect Old World mammals and are most prevalent in bats and nonhuman primates. The life cycle of *Hepatocystis* differs to that of *Plasmodium* by the fact that no multiplication of parasites occurs in the blood. Furthermore, parasites of *Hepatocystis* develop macroscopically visible characteristic merocyst stages in the liver of their hosts, in which the parasites multiply asexually, resulting in large numbers of merozoites that are released into the blood stream. The parasite infections are considered as benign. A previous study analyzing the draft genome and transcriptome of a primate *Hepatocystis* species found evidence for the loss of blood schizogony in *Hepatocystis* parasites.

In this study we investigate the between-host heterogeneity in the patterns of gene expression in *Hepatocystis*-infected African fruit bats of the species *E. labiatus* in Northern Uganda. Blood, liver, and spleen samples were collected from 114 bats. We analysed 3´-Tag RNA-Seq data from liver and spleen tissues from *Hepatocystis*-blood stage positive and uninfected bats. Differential gene expression analyses were carried out to investigate potential differences in gene expression regarding season and sex, age, reproductive status, and infection status of the bat hosts. We aim to identify host genes that are differentially expressed in the liver and in the spleen and determine their functions and roles they might play in the bat host immune response to infections with *Hepatocystis* parasites and compare our findings to *Plasmodium* infections in mammals.

The preliminary data revealed a high number of differentially expressed transcripts (DETs) for the parameters host age and breeding status. The letter could point to an impact of *Hepatocystis* infections on the host during periods of stress (such as breeding season). Higher numbers of DETs were also recovered in the spleen tissue, where immune genes in the host are active. Interestingly, blood stage parasitemia did not correlate with host gene expression, neither in the liver nor in the spleen.

P37

Analyzing the invasion process of *Entamoeba histolytica* through the intestinal tissue using an organoid derived 2D monolayer model.

J. Anders[1], A. Müller[2], D. Holthaus[2], C. König[1], C. Klotz[2], I. Bruchhaus[1]

[1]BNITM, RG Host Parasite Interaction, Hamburg, Germany
[2]RKI, RG Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany

Juliett Anders[1], Antonia Müller[2], David Holthaus[2,] Constantin König[1], Christian Klotz[2], Iris Bruchhaus[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Host Parasite Interaction, Hamburg, Germany; [2]Robert Koch Institute, Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany

Introduction

Entamoeba histolytica is a protozoan parasite which causes the disease amoebiasis in humans. In 90 % of infections, the parasite persists in the intestine without causing symptoms, with the amoebae becoming invasive in 10 % of cases. *E. histolytica* can reach other organs, mainly the liver, through the bloodstream and form abscesses there (1 % of cases). If left untreated, theses abscesses can lead to the death of the host. The reasons why *E. histolytica* is sometimes invasive and sometimes non-invasive are not yet fully understood. To get a better insight into the processes behind the tissue invasion of *E. histolytica,* we are using an intestinal organoid derived 2D monolayer model.

Materials & methods

Organoids of the small and the large intestine were grown from intestinal crypts containing adult stem cells and maintained in culture. Organoid derived single cells were seeded onto a filter membrane in a transwell system, where they formed a monolayer after several days. The organoid derived monolayers were characterized via immunofluorescence assays (IFA) and via measurement of the transepithelial electrical resistance (TEER).

Infection of the organoid derived monolayers with the non-pathogenic A1 amoeba clone and with the pathogenic B2 amoeba clone was performed. The infection was analyzed using TEER measurement, IFAs and dual RNAseq.

Results

The data show a decrease in the TEER value within several hours of infection for both amoeba clones.

P38

DOT1L regulates the dynamics of the host chromatin during host – pathogen interaction

A. Kanojia[1], G. Bhrigu[1], R. Muthuswami[1]

[1]Jawaharlal Nehru University, School of Life Sciences, New Delhi, India

Visceral Leishmaniasis ia caused by *L. donovani.* Existing drugs show severe side effects and drug resistance, therefore, new effective treatments against leishmaniasis are needed. In recent years, epigenetic modulators have emerged as attractive drug targets.

Mammalian DOT1L contains catalytic domain region, which can potentially methylate H3K79, which involved in cell cycle progression. However, the role of DOT1L during host pathogen interaction has not yet been delineated. Therefore, we undertook studies to explore the role of this epigenetic modulator during infection.

We have infected the differentiated THP1 macrophage cell line with leishmania and then check the mRNA level and protein level of DOT1L. We have also check the methylation activity of DOT1L as well as the protein expression of histone methylation marks after infection. Also we did the knockdown studies of DOT1L and then determine the parasitemia load. We also checked the mRNA level of defensins gene.

It been observed that the mRNA level of DOT1L increases on 6h of infection as compared to 0 hour of infection which leads to increase in protein expression of H3K79 methylation . Silencing of DOT1L decreases the methylation activity of DOT1L and so the parasitemia load indicating methylation mediated by DOT1L is required for *L. donovani* infection. To understand the mechanism, we analysed the expression of defensins genes in siDOT1L cells and found them to be upregulated as compared to cells transfected with scrambled RNA. Based on these results, we hypothesise that H3K79 methylation mediated DOT1L is required for downregulation of defensins gene on *Leishmania* infection.

So we conclude that our results indicate that DOT1L expression is required for *Leishmania donovani* infection and therefore, might be an potential drug target for the treatment of Leishmaniasis.

P39

Insights on the host response to *Cryptosporidium parvum* infection

M. Dale[1], C. Borrelli[2], A. Naguleswaran[1], A. Moor[2], P. Olias[3]

[1]University of Bern, Institute of Animal Pathology, Bern, Switzerland
[2]ETH Zürich, Department of Biosystems Science and Engineering, Zürich, Switzerland
[3]Justus-Liebig-University Gießen, Institute for Veterinary Pathology, Gießen, Germany

Diarrhoeal diseases are responsible for 10% of child deaths worldwide, making them the second leading cause of child mortality. The zoonotic parasite *Cryptosporidium parvum* is a major contributor to high diarrhea-related mortality rate in children and juvenile animals. Nitazoxanide is currently the only FDA-approved drug that shortens the duration of illness in immunocompetent human patient, but unfortunately is not better than a placebo in young children. The life cycle of the parasite occurs in a single host and includes the asexual and sexual stages. For the fecal-oral transmitted parasite, the host cells of the intestinal epithelium are crucial for development and replication. Currently, little is known about the host response to infection. We analyzed the transcriptome to study the dynamics in host cells related to infection. Several time points were chosen after infection throughout the life cycle of the parasite. Interestingly, we were able to observe dramatic changes on the transcriptional level over time. These results could form the basis for a better understanding of the host response that contributes to the severe intestinal pathology caused by the parasite.

P40

Characterization of extracellular vesicles derived from endothelial cells after co-incubation with *Plasmodium falciparum*-infected erythrocytes

M. D. P. Martinez Tauler[1], Y. Wu[1], B. Honecker[1,2], H. Torabi[1], K. Höhn[3], I. Bruchhaus[1], H. Heine[4], N. G. Metwally[1]

[1]Bernhard Nocht Institute, Protozoology, Hamburg, Germany
[2]Bernhard Nocht Institute, Molecular Infection Immunology, Hamburg, Germany
[3]Bernhard Nocht Institute, Imaging, Hamburg, Germany
[4]Research Center Borstel, Immunity, Borstel, Germany

Cerebral malaria (CM) affects 1% of malaria patients and is considered the most severe form of malaria. It primarily affects children under 5 years of age, is nearly 100% fatal without treatment, and often leaves survivors with severe cognitive deficits. CM pathology is characterized by a disruption of normal vascular function of the blood-brain barrier (BBB) upon adherence of *Plasmodium falciparum*-infected erythrocytes (IEs). To date, cytoadhesion of IEs was thought to be the main cause of BBB dysfunction. However, recent research suggests that also the activation of endothelial cells (ECs) is responsible for this severe form of pathology. The dynamics between the host immune system and malaria parasites is complex, and little is known about the immune evasion mechanisms of *P. falciparum*. Extracellular vesicles (EVs) may play a crucial function in disease progression, as they are essential for cell-cell communication and carry important genetic information such as microRNAs from the progenitor cell to the receiving cell. During malaria infection, the amount of EVs secreted by ECs increases significantly, which coincides with the case severity, indicating a key role for EVs during malaria pathogenesis. The purpose of this study is to investigate how infection with *P. falciparum* influences EVs production from human brain ECs and how they help malaria parasites to evade the immune system.In a first step EVs should be isolated by differential centrifugation and characterized using electron microscopy, nanoparticle tracking analysis, flow cytometry and miRNA sequencing. Using differential centrifugation, we were able to isolate EVs from brain ECs. Here, exosomes (100,000 x g, size 30-100 nm) and microvesicles (18,000 x g; size 50-200 nm) could be isolated separately from each other. Furthermore, stimulation of ECs by TNF? significantly increased the secretion of EVs.In addition, there is evidence that when stimulated with IFNɣ, an important cytokine during malaria infection, no significant change in EV production was observed. Therefore, EVs could be a potential target to enhance the immune response to P. falciparum and prevent the spread of infection.

P41

Genome-wide CRISPR screens for the identification of essential host factors for *Toxoplasma gondii* infection

A. G. Valenti[1], A. Naguleswaran[1], S. Rottenberg[1], P. Olias[1,2]

[1]Institute of Animal Pathology, Bern, Switzerland
[2]Institute for Veterinary Pathology, Gießen, Germany

Toxoplasma gondii is an apicomplexan parasite infecting virtually all warm-blooded animals, including birds. Roughly one fourth of the human population is chronically infected with this parasite. While mostly asymptomatic, chronic toxoplasmosis can exacerbate as a life-threatening acute condition in immunocompromised adults, such as people living with untreated AIDS and chemotherapy patients. From a veterinary point of view, *Toxoplasma* is also a parasite of significant economic burden, causing major losses in livestock production mainly due to abortion in small ruminants. Currently, none of the treatments available are sufficient to eliminate quiescent tissue cysts and eradicate the parasite from its host. Whilst to date most of the research on the molecular basis of the host-parasite interaction is focused on the parasite itself, little is known regarding the host factors that *Toxoplasma* requires for establishing infection. Notably, obligate intracellular parasites largely rely on the host cell, *e.g.* for the acquisition of essential metabolites and the release of toxic waste products. We are currently testing FACS-based genome-wide CRISPR/Cas9 knockout screens with the aim of identifying host factors that *Toxoplasma gondii* needs for intracellular development. Some of these metabolic factors may also be required by other parasites of the phylum Apicomplexa, constituting shared unifying fingerprints across different species. If successful, our results would broaden existing knowledge on host-pathogen interactions and open new possibilities to develop therapeutic approaches to treat toxoplasmosis by specifically targeting the host instead of the parasite.

P42

Effects of Dietary Fiber Supplementation on Host Immune Responses and Host Gut Microbiome in Roundworm-infected pigs

P. Höfler[1], M. Ankur[1], S. Josephine[1], H. Susanne[1]

[1]Freie Universität Berlin, Insitute of Immunology, Berlin, Germany

Ascaris is one of the most prevalent intestinal parasites in humans and pigs causing stunting and emaciation in infected individuals. For pig farmers this leads to serious economic losses.

The aim of thisl project is to investigate a potential beneficial influence of dietary fibers on an infection with *Ascaris suum* in pigs.

Dietary fibers increase the viscosity of the ingesta and thereby enlarge the mucus layer. As a consequence, we hypothesize that less larvae penetrate the cecum after infection to start the body migration. In addition, we hypothesize that the helminth-induced regulatory type 2 immune response will be enhanced by the bacterial metabolites derived from the dietary fibers. In particular, we expect short chain fatty acids (SCFA) as metabolites which get fermented by the gut microbiota. SCFAs were shown to activate gut dendritic cells and to induce polarization of Th2 cells which subsequently supports worm clearance. In parallel, we expect increased regulatory T cells which dampen inflammatory processes.

The experimental approach will investigate an alternative method to anthelmintic therapy and vaccination by administering a diet with inulin and sugar beet pulp as a new approach to modulate worm burden and severity of symptoms caused by infection with *A. suum* in pigs. Readouts will include diet-associated changes of the immune response, the gut microbiome, and infection efficacy in the natural host, the pig.

First *in vitro* approaches using gas chromatography have detected four different SCFAs in the excretory/secretory products of *A. suum.* In addition to assessing SCFA production by the parasite, we are examining what effect SCFAs have on porcine PBMC versus mesenteric lymph node derived dendritic cell and monocyte cytokine production. Preliminary data in monocytes confirm the trend that SCFA incubation lead to a decreased tumor necrosis factor production.

P44

Paving the way for the introduction of paediatric praziquantel in schistosome endemic countries: A cross-sectional study from Madagascar

A. Guth[1], D. Koecher Andrianarimanana[2], M. Rasamoelina[3], V. Pavoncello[1], P. Rausche[1], C. Doumbia[1], A. Remkes[1], A. Zafinimampera[4], O. Totofotsy[4], S. R. Rakotomalala[5], J. May[1,6], V. Marchese[1,6], R. A. Rakotoarivelo[7], D. Fusco[1,6]

[1]Bernhard Nocht Institute for Tropical Medicine, Infectious disease, Hamburg, Germany
[2]Centre Hospitalier Universitaire, Department of Pediatric, Mahajanga, Madagascar
[3]Centre d'Infectiologie Charles Mérieux (CICM), Antananarivo, Madagascar
[4]Centre Hospitalier Universitaire, Mahajanga, Madagascar
[5]Centre Hospitalier Universitaire, Department of Clinical Biology, Mahajanga, Madagascar
[6]German Center for Infection Research (DZIF), Hamburg, Germany
[7]University of Fianarantsoa, Department Infectiology, Fianarantsoa, Madagascar

Background: Under 5 years old children are a vulnerable population burdened with several diseases. Schistosomiasis, a parasitic disease endemic in many low- and middle-income country (LMIC) and especially in Africa affects mostly children. Recent studies are showing high prevalence of schistosomiasis among young children of pre-scholar age. Mass drug administration, the most used strategy to prevent and control the disease, is addressed to school-aged children hence systematically excluding preschool-age children (PSAC). To close this treatment gap a new paediatric drug formulation has been carried out by the pediatric praziquantel consortium and is actually going into an European Medicines Agency"s application.

The objective of our study is to assess the feasibility of PSAC treatment for schistosomiasis in a highly endemic area.

Method: This cross-sectional study is implemented in the regions of Boeny and Haute Matsiatra of Madagascar. A Praziquantel treatment will be proposed to the caregivers of 5000 children aged from 9 to 24 months. Acceptance, refusal and tolerability will be assessed. Quantitative and qualitative data will be collected from caregivers and health care workers to assess acceptability and feasibility of the intervention. Statistical analysis will be performed by means of MAXQDA and R.

Results: A total of ten different sites have been selected to perform our study. The beginning of the treatment is planned for February 2023 for a total duration of five months. The investigation tool has been finalised and tested for use in the area of the study.

Conclusion: Our study will provide essential information to facilitate the introduction of the paediatric praziquantel in Madagascar after its marketing. We hope to decrease the phase between marketing and in country implementation of the drug in order to contribute to the 2030 NTD road map which targets the elimination of schistosomiasis as a public health problem.

P45

The puzzle of the acanthocephalan male reproductive organs and their role in copulation and sexual selection

B. Sayyaf Dezfuli[1], F. Pironi[1], A. Lussu[1], G. Bosi[2]

[1]University of Ferrara, Life Sciences & Biotecnology, Ferrara, Italy
[2]Università di Milano, Health, Animal Science and Food Safety, Milan, Italy

Introduction

Reproduction of the Acanthocephala always received less attention. Many unanswered questions and mysteries on some processes in male and female reproductive apparatus. Among some taxa of invertebrates, such as the Insecta and Acanthocephala, several behavioral and physiological adaptations of males may help to avoid the second inseminations of the same female. One of these adaptations is a copulatory cap or chastity belt produced by the male accessory gland secretions as a plug within the female genital apparatus. Acanthocephalan male reproductive system includes testes, vas efferentia, seminal vesicle, cement glands, cement reservoir, Saetttigen"s pouch, copulatory bursa and penis. One of the most distinctive elements in the genital apparatus of male acanthocephalans are cement glands and their products have considerable importance in the reproductive process.

Objectives

Here we present the aspect of cement glands of species belonging to 4 classes of this phylum. Moreover, due to lack of information on relationship between different parts of male reproductive system herein ultrastructure of cement reservoir, seminal vesicle and Saefftigen"s pouch will be detailed.

Materials & Methods

From the intestine of fish and bird definitive hosts of different acanthocephalan species several males were selected and dissected and the whole genital apparatus were fixed for light and electron microscopy.

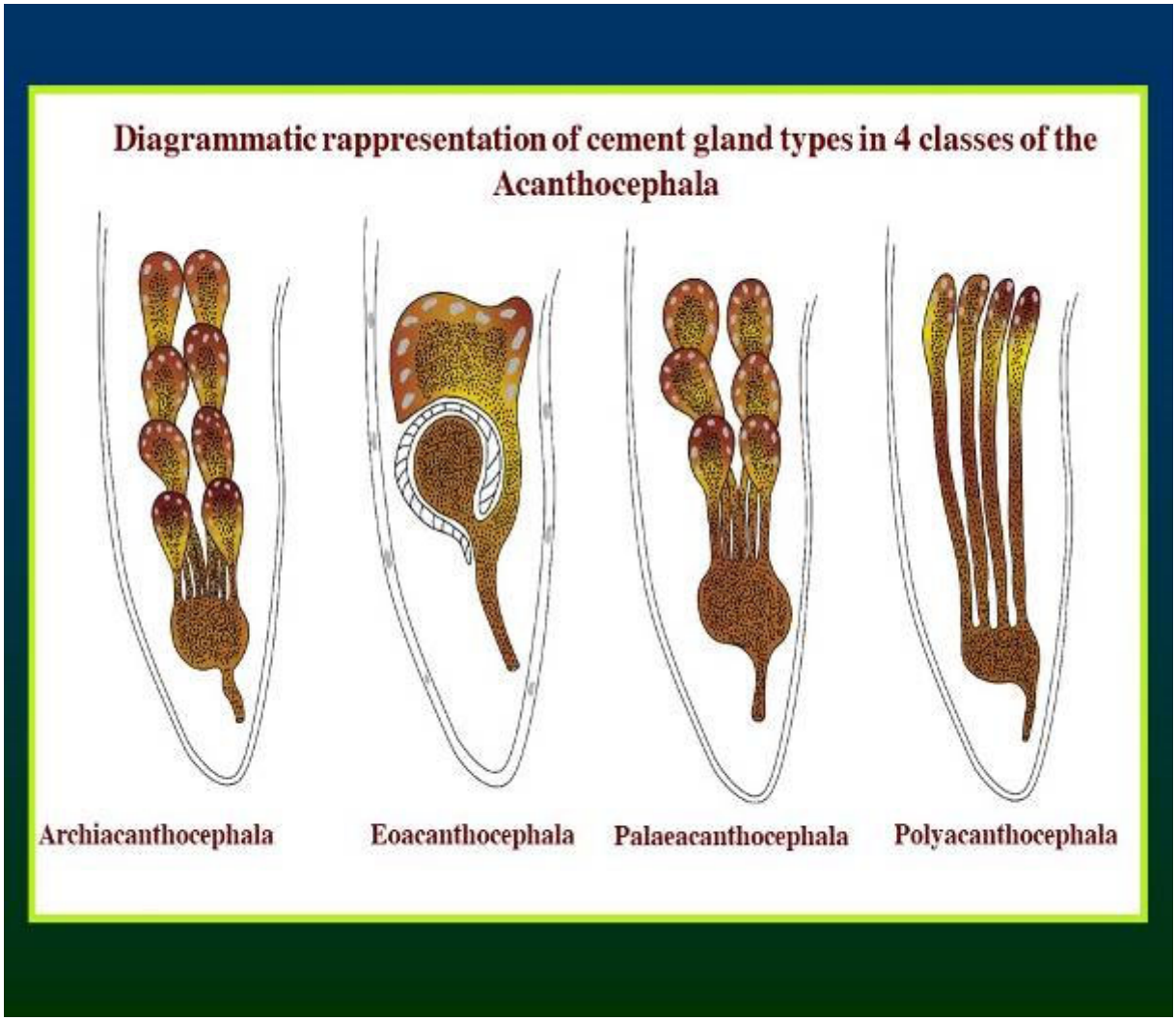
Results

In Acanthocephala cement glands vary in shape, number and arrangement in different classes of the taxon, generally each gland has an outer cytoplasmic layer containing nuclei and surrounding a space for storage of the cement material within the gland. The nuclei have an irregular outline and the cytoplasm of the cells contains round, membrane-bound secretory granules. Current study documented the fine structure of Saetttigen"s pouch and close contact between this organ and seminal vesicle, cement reservoir.

Conclusion

All species of Acanthocephala are polygamous, the capping behaviour evolved in response to sexual selection and such a cap will delay or prevent the introduction of sperm by another male. No work has been carried out on the ultrastructure of different organs of male reproductive apparatus. We report herein the first photographic evidence on ultrastructure of the Saetttigen"s pouch and its close contact with copulatory bursa, this organ is essential for extrusion of the male copulatory bursa.

Fig. 1


Diagrammatic rappresentation of cement gland types in 4 classes of the Acanthocephala
Archiacanthocephala
Eoacanthocephala
Palaeacanthocephala
Polyacanthocephala

P46

Effect of filarial lymphedema treatment with doxycycline on immune activation and exhaustion frequencies in blood

S. Horn[1], A. Feichtner[1,2], I. Kroidl[1,2], A. Ngenya[3], L. Masagati[3], J. Osei-Mensah[4,5], V. Serwaa Opoku[5,6], L. Batsa Debrah[5,6,7], U. Klarmann-Schulz[8,9,10], J. Kuehlwein[8], A. Kellings[11], M. Hoelscher[1,2], A. Kalinga[3], A. Y. Debrah[5,6,7,12], A. Hoerauf[8,9,10]

[1]Klinikum der Universität München, Department of Infectious Diseases and Tropical Medicine, Munich, Germany
[2]German Center for Infection Research (DZIF), Partner site, Munich, Munich, Germany
[3]National Institute for Medical Research (NIMR), Dar es Salaam, Tanzania, United Republic Of
[4]Department of Pathobiology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[5]Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana
[6]Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[7]German-West African Centre for Global Health and Pandemic Prevention (G-WAC), Kumasi, Ghana
[8]Institute of Medical Microbiology, Immunology, and Parasitology, University Hospital of Bonn, Bonn, Germany
[9]German Center for Infection Research (DZIF), Partner site, Cologne/Bonn , Bonn, Germany
[10]German-West African Centre for Global Health and Pandemic Prevention (G-WAC), Bonn, Germany
[11]Institute for Clinical Chemistry and Clinical Pharmacology, University Hospital of Bonn, Bonn, Germany
[12]Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Introduction:

Lymphatic filariasis is a mosquito-transmitted helminth infection caused by *Wuchereria bancrofti* and *Brugia* species. About 30% of the 68 million infected people worldwide suffer from disfiguring pathology (e.g. lymphedema). Current treatment (200 µg/kg ivermectin (IVM) and 400 mg albendazole (ALB)) acts on microfilariae, the larval stage that is also responsible for transmission, but does not affect the adult worm. Prior studies have shown 100-200mg Doxycycline to act not only on the adult worm, but also when taken daily for 6 weeks, lead to improvement of those with early stages of lymphedema.

Materials & Methods:

A double-blind, randomized, placebo-controlled trial (LEDoxy) was conducted in Ghana and Tanzania in order to confirm the effect of 200 mg Doxycycline treatment and to test the effect of 100 mg Doxycycline per day for 6 weeks on improvement of filarial lymphedema in study participants. Participants were characterized at baseline using the Dreyer staging (Dreyer *et* al., 2002), circumference measurements, and the LymphaTech® infrared scanner. Over a 24-month period, changes in pathology and immunological parameters were measured (end of treatment, 6 and 24 months post-treatment). Additionally, a novel flow cytometry based whole blood method developed by us was used to characterize CD4 and CD8 T cells for a number of activation, maturation, and exhaustion markers (CD45, CD27, FoxP3, CD25, CD38, HLADR, Tbet, Eomes).

Results – Conclusion:

Flow cytometry data from the first 20 Ghanaian and 43 Tanzanian participants who completed treatment has been analyzed. When comparing individual pre- and post-treatment data from Tanzania, we see no significant difference in the frequency between various T cell subgroups (central and effector memory CD4+ T cells). Yet, we observe an overall significant decline in immune activation parameters (HLADR+/CD38+ on CD4+ T cells) over the course of treatment. Interestingly, the opposite is observed in the Ghanaian samples when examining paired values from pre- and 6 months post-treatment. Clearer conclusions as to the nature of these differing results will be able to be drawn after unblinding of the study participants.

P47

Development of a novel rapid point-of-care PCR test for schistosomiasis

T. Josten[1], S. Abdellatif[1], Y. Hamway[1], C. Prazeres da costa[1]

[1]Technical University Munich (TUM), Institute for Medical Microbiology, Immunology and Hygiene, Munich, Germany

Introduction:

The WHO has targeted schistosomiasis for elimination as a public health concern in this decade. This requires, amongst other things, an improvement in diagnostics, as current approaches are not sufficiently sensitive and specific to achieve these goals, especially in low endemic areas.

Objectives:

We are developing a rapid test on a new platform, produced by a local startup. The aim will be to have a sensitive, specific and fast (10-20 minutes to result) PCR assay for field deployment to assist with elimination of the disease. In addition, we will compare different DNA extraction methods in order to have a user-friendly sample-to-answer process.

Materials and methods:

For this project we are working with a new technique, Pulse Controlled Amplification (PCA). The new platform is quite similar to a typical qPCR but with a new approach to nucleic acid amplification. It is based on a magnetic bead technology, which allows for a greater number of cycles in a shorter amount of time. For the detection we are using a novel primer-probe assay for *Schistosoma haematobium* and *mansoni*.

Results:

We have developed a PCR assay with high sensitivity and specificity on a platform that offers rapid, point-of-care testing capacity. We were able to perform the first successful runs with human serum samples. In the next steps, we will further expand on these results and compare them to existing methods such as antigen tests and egg counts.

Conclusion:

A novel, rapid point-of-care test for schistosomiasis such as this provides a siginifcant step towards overcoming current diagnostics limitations and towards achieving the WHO goal of eliminating the disease as a public health concern. Much work remains in order to optimize and validate this test, but it is promising as a completely new approach to tackling the issues at hand.

P48

National Research Data Infrastructure for the Research of Microbiota (NFDI4Microbiota)

C. Hege[1], C. Paulmann[1], A. Becker[2], P. Brock[3], T. Clavel[4], U. Nunes da Rocha[5], K. U. Förstner[6], A. Goesmann[7], B. Götz[6], M. Menz[8], J. Overmann[9], J. Stoye[10], A. McHardy[1]

[1]Helmholtz Centre for Infection Research GmbH, Brunswick, Germany
[2]Philips-Universität Marburg, Marburg, Germany
[3]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
[4]RWTH Aachen University, Aachen, Germany
[5]Helmholtz Centre for Environmental Research, Leipzig, Germany
[6]ZB Med - Information Centre for Life Science, Cologne, Germany
[7]Justus-Liebig-University Gießen, Gießen, Germany
[8]Friedrich-Schiller-Universität Jena, Jena, Germany
[9]German collection of Microganism and Cell Cultures (DSMZ), Brunswick, Germany
[10]Bielefeld University, Bielefeld, Germany

Introduction

Recent technologies like high-throughput molecular sequencing lead to the generation of large data. However, the use and re-use of this data has failed to exploit its potential. The NFDI (National Research Data Infrastructure) wants to change this by developing a comprehensive research data management, encompassing different consortia. NFDI4Microbiota aims to facilitate the digital transformation in the microbiological community (bacteriology, mycology, virology and parasitology). Providing access to data, analysis services, training and standards.

Objectives

Central for the NFDI4Microbiota consortium is the development and provision of the computational infrastructure and analytical workflows required to store, access, process, and interpret various microbiome- and parasitology-related data types. NFDI4Microbiota works on developing and implementing software and standardized workflows for users to analyse their own datasets (i.e. for quality control, data processing, statistical analyses, and visualizations of different data types and results).

Materials & methods

The German microbial research will be engaged through training and community building activities, and by creating a cloud-based system that will make the storage, integration and analysis of microbial data, especially omics data, consistent, reproducible, and accessible. So, NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable and Re-usable) principles and Open Science.

Results

NFDI4Microbiota consists of ten well-established partner institutions, is supported by five professional societies and more than 50 participants. Several workshops and training events for the community have already taken place and more will follow. Moreover, the consortium launched an ambassador program to connect with the participants, thereby helping to identify the needs of their local community. Technical solutions are developed, tested and refined in several use cases from different fields of microbiology. All relevant information and specific services are made available via the web portal.

Conclusion

Producers and users of data will benefit from FAIR data (Findable, Accessible, Interoperable and Re-usable) more likely to be cited and integrated into a wider microbial inquiry. The current data parasitism would shift to a future data mutualism benefiting all partners. The NFDI4Microbiota will support the parasitology community through this process with an elaborate training program.

Fig. 1

P49

Trypanosoma brucei brucei-induced aggregated NET formation depends on P2X1 and P2Y6 purinergic receptors

D. Grob[1], C. Larrazabal[2,1], T. Vellmer[3], C. J. Janzen[3], U. Gärtner[4], A. Taubert[2,1], C. Hermosilla[2,1], I. Conejeros[2,1]

[1]Institute of Parasitology, Gießen, Germany
[2]Justus-Liebig-Universität Gießen, Gießen, Germany
[3]University of Würzburg, Department of Cell & Developmental Biology, Biocenter, Würzburg, Germany
[4]Justus-Liebig-Universität Gießen, Institute of Anatomy and Cell Biology, Gießen, Germany

Introduction

Trypanosoma b. brucei is an euglenozoan parasite that is able to infect a wide range of hosts, including humans and bovines. In cattle, *T. b. brucei* is one of the pathogenic agents causing Animal African Trypanosomiasis (AAT) or Nagana disease. *T. b. brucei* life cycle is indirect, with the tsetse flies acting as vectors. In the vertebrate host, *T. b. brucei* multiplies extracellularly as trypomastigote forms in the host blood, thereby directly being exposed to cells of the innate immune system, such as polymorphonuclear neutrophils (PMN). PMN own several innate effector mechanisms to combat invading pathogens, including neutrophil extracellular trap (NET) formation.

Objectives

To study early interactions between bovine PMN and *T. b. brucei* trypomastigotes, PMN activation and NET formation. The role of P2X1 and P2Y6 purinergic receptors in trypomastigote-driven NETosis was also evaluated.

Materials & methods

T. b. brucei trypomastigotes were cultured in HMI-79 medium. Bovine PMN were isolated from whole blood with hypotonic lysis of erythrocytes. PMN activation was evaluated on the level of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Percentages of PMN forming NETs were studied by scanning electron, epifluorescence and live cell 3D holotomographic microscopy. For positive controls, PMN were treated with ionomycin or monosodium urate (MSU) crystals. To block P2X1 and P2Y6 purinergic receptors, PMN were treated with the inhibitors NF449 and MRS2578, respectively, before parasite exposure.

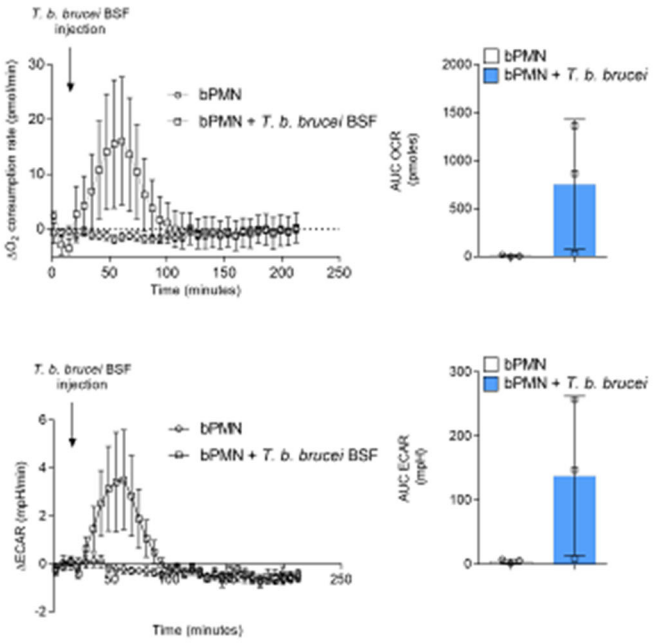
Results

The exposure to *T. b. brucei* typomastigotes induced immediate activation of PMN as indicated by increased OCR and induce a metabolic switch towards glycolysis in PMN as deduced from enhanced ECAR. PMN responded to parasite encounter via NET formation. *T. b. brucei* rypomastigotes predominantly induced *agg*NETs. Ionomycin or MSU also resulted in *agg*NET formation. Furthermore, *T. b. brucei* typomastigote-driven PMN activation and *agg*NET formation revealed as dependent on P2X1 and P2Y6 purinergic receptors since PMN pretreatments with NF449 and MRS2578 impaired all three mechanisms finally resulting in decreased OCR, ECAR and *agg*NET formation.

Conclusion

Exposure of PMN to *T. b brucei* trypomastigotes leads to PMN activation and triggering an enhancement in OCR/ECAR and *agg*NET formation. All these neutrophil functions depend on purinergic signaling pathways via P2Y6 and P2X1 receptors.

Fig. 1


T. b. brucei BSF injection
bPMN
bPMN + T. b. brucei BSF
Time (minutes)
AUC OCR (pmoles)
bPMN
bPMN + T. b. brucei
T. b. brucei BSF injection
bPMN
bPMN + T. b. brucei BSF
Time (minutes)
AUC ECAR (mpH)
bPMN
bPMN + T. b. brucei

P50

Bovine monocyte extracellular traps (METs) formed against *Cryptosporidium parvum* under physioxic and hyperoxic conditions

S. S. Hasheminasab[1], I. Conejeros[1], U. Gärtner[2], F. Kamena[3], A. Taubert[1], C. Hermosilla[1]

[1]Institute of Parasitology, Gießen, Germany
[2]Institute of Anatomy and Cell Biology, Gießen, Germany
[3]Department of Microbiology and Parasitology, Buea, Cameroon

Introduction: Cryptosporidiosis is caused by the zoonotic apicomplexan protozoa *Cryptosporidium parvum* infecting small intestinal epithelial cells. Referring to early host innate immune reactions during cryptosporidiosis, little is yet known. Nonetheless, previous studies showed that human and bovine NETosis – an important host innate effector mechanism of polymorphonuclear neutrophils (PMN)- is elicited by *C. parvum* stages (Muñoz-Caro et al., 2015). Conversely to PMN, very little is known on monocyte-derived effector mechanisms against this enteric parasite. **Objectives:** Aim of the current study was to investigate the release of monocyte extracellular traps (METs) against two *C. parvum* stages, i. e. oocysts and sporozoites, under physioxia and hyperoxia. Additionally, the role of monocarboxylate transporters (MCTs), Notch pathway signalling and purinergic signalling via the P2X1 receptor for *C. parvum*-triggered METosis was evaluated. **Materials and methods:** *C. parvum* oocysts and sporozoites were exposed to bovine monocytes to investigate METs under hyperoxic (21% O2) and intestinal physioxic (5% O2) conditions. For the detection of *C. parvum*-triggered METosis, immunofluorescence, confocal, 3D holotomographic and scanning electron microscopic analyses were performed. The role of MCT1, MCT2, P2X1 and Notch pathway in *C. parvum*-mediated METosis was studied via functional inhibition assays by pretreating monocytes with the inhibitors AR-C141900, ARC155858 and NF449, Compound E and DAPT, respectively. **Results:** Exposure of monocytes to both parasite stages resulted in suicidal METosis as confirmed by SEM and Live cell 3D holotomographic microscopy. Immunofluorescence and confocal microscopic analyses unveiled that METs were capable to entrap parasites. Pre-treatments of monocytes with MCT inhibitors reduced MET formation after parasite exposure under both oxygen conditions, but not significantly. The same held true for NF449 pre-treatments resulting in the barely significant reduction of *C. parvum*-triggered METosis. Regarding Notch pathway, reduction was not significant in both oxygen conditions, but it seems that Compound E is more relevant to METosis **Conclusions:** Exposure of *C. parvum* sporozoites and oocysts to bovine monocytes leads to suicidal METosis under physioxia and hyperoxia. Overall and in contrast to neutrophil-mediated reactions,P2X1- and MCT-mediated pathways seem of minor importance in *C. parvum*-induced suicidal METosis.

P51

First report of *Dirofilaria immitis* infection from dogs in Kinshasa, Democratic Republic of Congo

M. Kapanga[1]

[1]University of Kinshasa, Faculty of Veterinary Medicine, Kinshasa, Congo, The Democratic Republic Of The

INTRODUCTION Dirofilaria immits is a mosquito-borne filaroid helminth that affects domestic and wild carnivores, causing heartworm disease associated whith respiratory complications. This parasite is also known to cause a zoonotic (pulmonary dirofilariosis) and an emerging disease of wich dog can serve as the main animal reservoir. Little information is reported in Africa while the disease tends to spread in other continents. In Democratic Republic of Congo, dirofilariosis was not previously documented. OBJECTIVES The aim of this study was to confirm the presence of D. immitis in dog and to determine its prevalence in some areas of Kinshasa. MATERIELS ET METHODS A preliminary survey was conducted in 6 main illegal dog slaughter sites located in 6 of the 24 districts of Kinshasa. Of the total of 77 dogs (over ten months of age of different sexes) slaughtered, heart and lung were collected from each dog, transported and conserved at -20oC at the Laboratory of veterinary parasitology of the University of Kinshasa. After thawing, right heart was incised and analyzed for D. immitis adult worms. In all positive samples, worms were isolated, counted, measured and kept in 70% alcohol for future use. RESULTS In total, 4 samples were positive (P = 5.1%). Infestation ranged from one to a dozen of adult worms isolated from right atrium, right ventricle and pulmonary arteries. All positive samples came from two of the six slaughter sites. CONCLUSION This is the first report of D. immitis in Kinshasa. Besides animal health concerns, this situation must be considered as a public health threat and deserves special attention, in particular to identify the main risk factors for the transmission of this parasite in Kinshasa.

Fig. 1

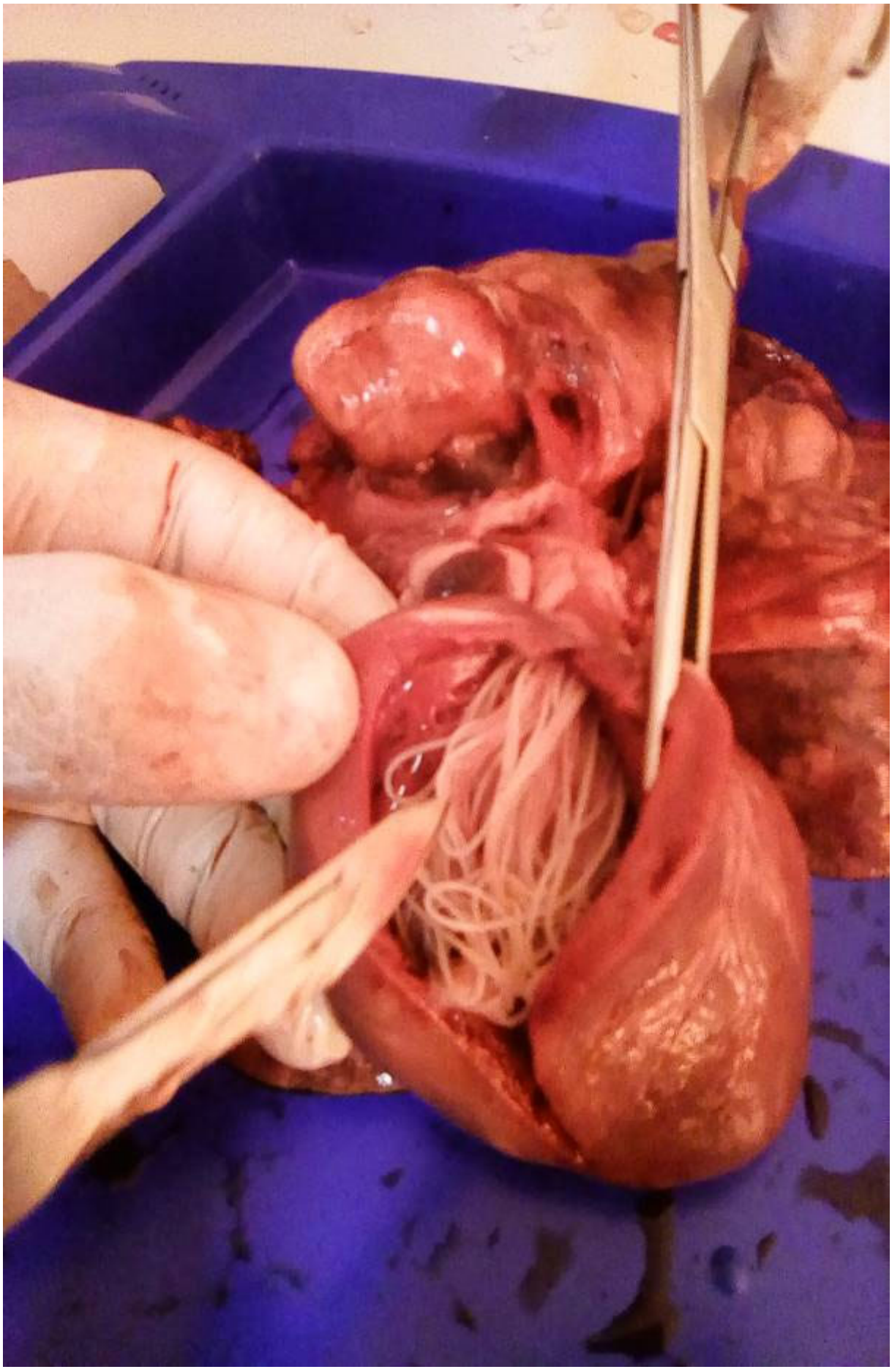
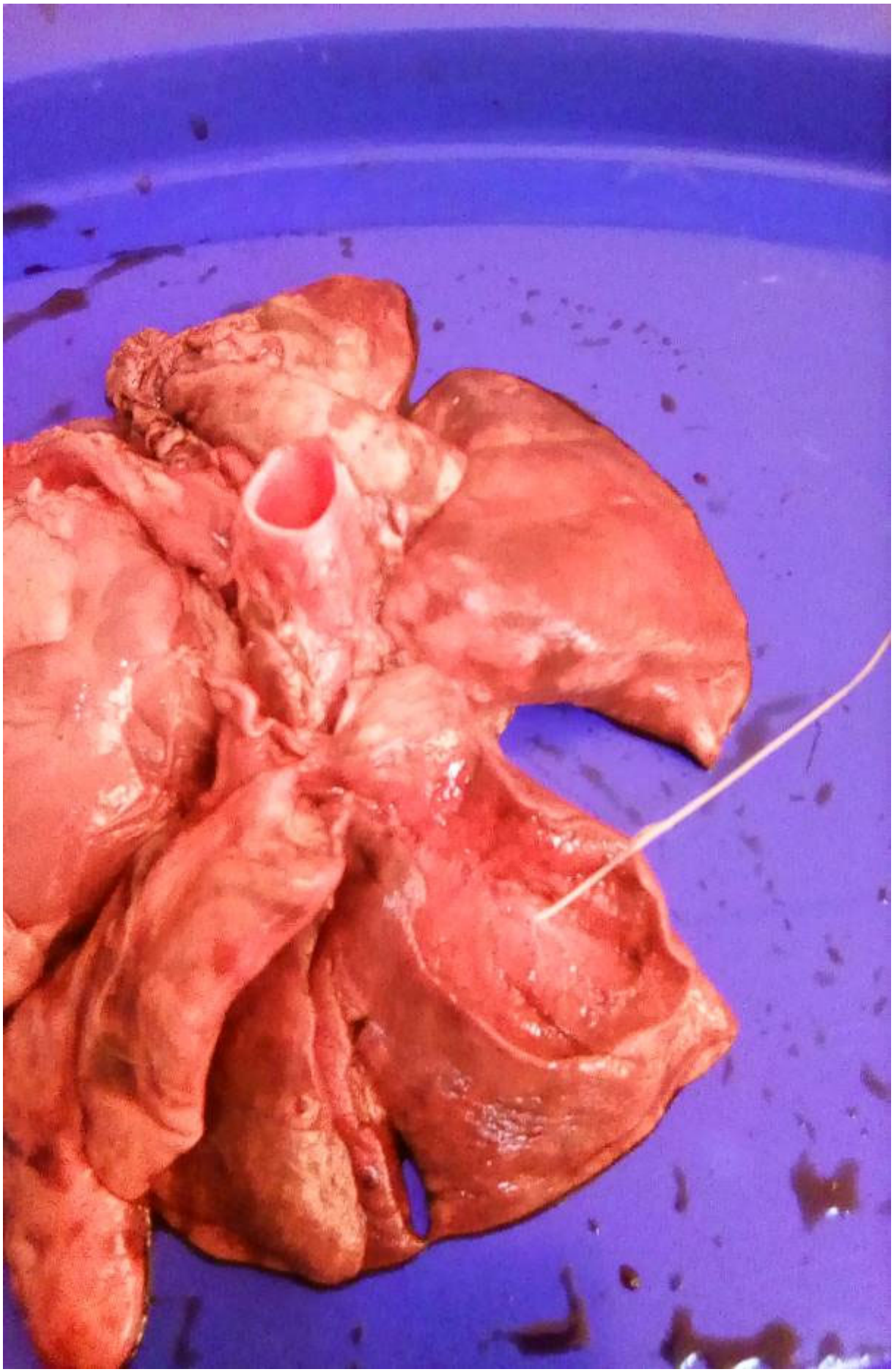


Fig. 2



P52

Toxoplasma gondii infection-driven genome instability is haplotype- and cell type-independent

L. Rojas Barón[1], I. Conejeros[1], S. Butterworth[2], M. Treeck[2], C. Hermosilla[1], A. Taubert[1], Z. Velásquez[1]

[1]Biomedical Research Center Seltersberg, Justus Liebig University Gießen, Institut für Parasitologie, Gießen, Germany
[2]The Francis Crick Institute, Signalling in Apicomplexan Parasites Laboratory, London, United Kingdom

Introduction: *Toxoplasma gondii* is an obligate intracellular coccidian parasite, which is well known for its extraordinary capacity to modulate its host cell. The three main *T. gondii* clonal lineages exhibit different degrees of virulence. Thus, type 1 strains lead to widespread parasite dissemination and death in mice, whilst type 3 is considered avirulent. As recently demonstrated, *T. gondii* infections affect host cell cycle progression, chromosome segregation and cytokinesis, all hallmarks of genome instability triggered by genomic DNA damage.

Objective: We here studied whether *T. gondii*-driven genome instability is a parasite strain (haplotype)- or cell type-dependent event.

Materials & Methods: We here compared infections with *T. gondii* RH, Me49 and NED strains representing, type 1, 2 and 3 haplotypes. FACS-based analyses on cell cycle progression were performed in different primary human and bovine cell types (e. g. HUVEC, HFF and BUVEC). Both, immunofluorescence assays detecting ᵞH2Ax-related DNA damage foci and comet assays were used to estimate DNA damage in *T. gondii*-infected cells and controls. Activation of the DNA damage repair pathway was studied by Western blot-based quantification of related key proteins. Since a main source of intracellular DNA damage is enhanced ROS, we also analyzed intracellular and extracellular ROS concentrations in *T. gondii*-infected BUVEC by DCF-DA and AmplexRed assays, respectively.

Results: In line with recent observations on the RH strain, all cell types used were found arrested in S-phase showing increased proportions of binucleated cells. These findings were strain-independent since they also were observed in *T. gondii* NED and Me49 infections. In addition, all three haplotypes induced DNA strand breaks within 24 h p. i. Moreover, RH strain was shown to activate the host cell homologous recombination repair pathway. In addition, exclusively the first step of the non-homologous end-joining pathway was activated in host cells after 24 h p. i. Overall, no changes in intra- or extracellular ROS were detected in any infection.

Conclusion: Our data suggest that *T. gondii* infection-driven alteration of host cell cycle progression and cytokinesis is independent of the host cell type, host species origin and parasite haplotype. In addition, both *T. gondii*-driven host cellular DNA strand breaks and activation of the homologous recombination pathway occurred independent of host cellular ROS production.

P53

Assessing the *in vitro* efficacy of native *Mucor* sp. isolates against *Trichuris* sp. eggs from Captive Non-Human Primates

M. Lourenço[1,2], J. Lozano[1,2], L. Gomes[1,2], R. Bernardino[3], M. Oliveira[1,2], A. Paz-Silva[4], L. Madeira de Carvalho[1,2]

[1]CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal
[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisboa, Portugal
[3]Lisbon Zoo Veterinary Hospital, Lisboa, Portugal
[4]Control of Parasites Research Group (COPAR, GI-2120), Department of Animal Pathology, Faculty of Veterinary, University of Santiago de Compostela, Lugo, Spain

Introduction: Nematodes of the genus *Trichuris* are gastrointestinal (GI) parasites of high clinical importance in captive non-human primates (NHPs) kept at zoological institutions, being difficult to control due to anthelminthic drug resistance and eggs" survival in the environment. However, sustainable integrated control approaches are being developed, namely the use of nematophagous fungi.

Objectives: In this study, the ovicidal activity of five native *Mucor* sp. isolates was tested *in vitro* against *Trichuris* sp. eggs.

Materials & methods: A total of five native *Mucor* sp. isolates (MBB1, MBB2, MDM, ML, and MSM), previously obtained from faecal samples of different NHP groups of the Lisbon Zoo (Portugal), were used in this research. Two different assays were performed: in Petri dishes (PD), and in a faecal microenvironment (FM) model using Hamadryas Baboon faeces containing *Trichuris* sp. eggs. Control samples with no fungi inoculation were used in both assays. Each sample was observed once or twice a week, on the FM and PD assays, respectively, and isolates ovicidal activity was characterized and quantified, with the proportions of viable and unviable eggs being compared between each isolate and the control group, using the Fisher"s Exact Test (95% confidence). Eggs showing capsule deformation or even rupture, were considered unviable. Both assays lasted three weeks.

Results: In the PD assay, samples inoculated with MBB1, MBB2, MDM, ML, and MSM isolates had ovicidal efficacies of 50%, 0%, 43%, 54%, and 100%, respectively, after three weeks of exposure. Also, at the end of the FM assay, these isolates had efficacies of 14%, 12%, 4%, 14%, and 33%, respectively. All eggs in the control samples remained viable on both assays. Significant differences in eggs" viability were observed for the isolates MBB1, MDM, ML, and MSM on the PD assay, and only for the MSM isolate in the FM assay, in comparison with control.

Conclusion: These results suggest that the use of MSM *Mucor* sp. strain could provide a valuable complement to the standard deworming practices in NHPs, as an innovative and sustainable integrated approach for GI parasite control.

Funding: This research was funded by CIISA/FMV Project UIDB/00276/2020 and LA/P/0059/2020 - AL4AnimalS (both funded by FCT). Also, João Lozano owns a PhD research fellowship 2020.09037.BD (funded by FCT).

P54

Occurrence of *Prosthenorchis elegans* infections in neotropical primates of a conservation centre in the Amazon Basin of Peru

L. Segeritz[1], O. Conrads[2], D. Bakker[2], A. Taubert[1], C. Hermosilla[1]

[1]Justus-Liebig-University Gießen, Institute of Parasitology, Gießen, Germany
[2]Esperanza Verde Peru, Bello Horizonte, Ucayali, Peru

Introduction

Prosthenorchis elegans is a neglected intestinal acanthocephalan parasite, which infects primates by consuming arthropod intermediate hosts. Adult worms can perforate the intestinal wall of various primate definitive hosts causing lethal secondary peritonitis. So far, no medical treatment for prosthenorchiosis is available.

Objectives

The main objectives were to evaluate occurrence, pathology and clinical impact of *P. elegans* infections in various neotropical primate species in endemic areas under field conditions.

Materials & methods

P. elegans infections in primates originating from a conservation centre in the Amazon Basin in Ucayali, Peru (8° 27" 0.59""; W 75° 15" 11.518"") were analysed in 2014-2022 by coproscopy of faecal samples (direct smear, sedimentation, SAF and flotation technique). Moreover, succumbed primates (*n* = 40) were necropsied and molecular analysis of adult *P. elegans* specimen was performed.

Results

In total, 42.5 % (17/40) necropsied primates of six different species (*Lagothrix lagothricha*, *Pithecia aequatorialis*, *Leontocebus leucogenys*, *Aotus nancymaae*, *Sapajus macrocephalus*, *Ateles chamek*) were found to be infected with *P. elegans*. Additionally, prosthenorchiosis was detected by coprology in another primate species, the red howler monkey (*Alouatta seniculus*). To our knowledge, this study includes the first host records of *P. elegans* in saki monkeys (*Pithecia* sp.), black-faced black spider monkeys (*A. chamek*) and red howler monkeys (*A. seniculus*).

Conclusion

Due to the ubiquitous and abundant occurrence of invertebrate intermediate hosts in the tropical environment, a considerable expansion of *P. elegans* infections was stated, once it was introduced into the current conservational area. Overall, elimination of this parasitosis seems impossible by currently available means. Therefore, prosthenorchiosis still represents a significant threat and challenge in neotropical primate conservation programs and scientific attention to this topic is desirable. Challenges include the development of safe, specific and efficient anthelmintic drugs against *P. elegans.*

P55

Troglostrongylus brevior and other lungworm infections in Eurasian lynx (Lynx lynx) of the Harz Mountains, Germany

M. Haas[1], L. Segeritz[1], O. Anders[2], T. L. Middelhoff[2], A. M. Tun[3], A. Taubert[1], C. Hermosilla[1]

[1]Justus-Liebig-University Gießen, Institut for Parasitology, Gießen, Germany
[2]Nationalparkverwaltung Harz, Außenstelle Oderhaus, St. Andreasberg, Germany
[3]Justus-Liebig-University Gießen, Klinik für Schweine, Gießen, Germany

Introduction:

The formerly widely spread Eurasian lynx (*Lynx lynx*) nowadays represents an endangeredsmall felid species (Felinae), freely ranging in only three small fragmented populations in Germany. To date, little is known on lynx pathogens, especially in the field of cardio-pulmonary parasites. Moreover, data on the occurrence, pathology and clinical significance of lungworm infections in lynx are scarce. In domestic cats, metastrongyloid lungworms can induce different clinical signs ranging from subclinical courses to minor and severe symptoms like coughing, dyspnea, cardiac hypertrophy or even death.

Objectives:

The current study was initiated to study neglected metastrongyloid lungworm infections in re-introduced free-ranging *L. lynx* populations in Germany.

Material and Methods:

In 2022, sixteen faecal samples from seven[LS1] [MH2] GPS-collared sub-adult Eurasian lynxes from the Harz Mountain population were collected (at around 51.855380, 10.345010). Sampling was performed at natural prey killing sites in a non-invasive and un-molested manner. The fresh faecal samples were analyzed for vital metastrongyloid larvae using the Baermann funnel technique. First-stage lungworm larvae were identified microscopically based on classical morphological characteristics. To confirm morphological results on species level, molecular analyses were performed via PCR.

Results:

By coprological analysis, we here deliver the first report on *Troglostrongylus brevior* infection in German Eurasian lynx. The morphometric and morphological identification of *T. brevior*-larvae was confirmed by PCR and. Furthermore, larvae of other metastrongyloid lungworms, i. e. *Angiostrongylus* sp., *Aelurostrongylus abstrusus* and *Crenosoma* sp. were detected in lynx samples. Overall, a high proportion (56%) of lynx faecal samples proved positive for metastrongyloid cardiopulmonary nematode infections.

Conclusion:

Considering the high proportion of lungworm-positive faecal samples, further studies on metastrongyloid lungworm infections circulating in Eurasian lynx should be performed to better understand the epidemiology, prevalence and pathobiology of this parasite infection. The implementation of a veterinary monitoring program on *Lynx lynx* is pivotal to assess their health status and to succeed in conserving this endangered small wild felid species.

P56

Re-monitoring of a hyperendemic focus of *Angiostrongylus vasorum* infections in native gastropod populations in Southern Germany

A. Dusch[1], *L. Segeritz*[1], *A. Taubert*[1], *C. Hermosilla*[1]

[1]Justus-Liebig-Universität Gießen, Parasitology, Gießen, Germany

Introduction

The metastrongyloid nematode *Angiostrongylus vasorum* causes severe cardiovascular and pulmonary disease in domestic dogs. Geographically, canine *A. vasorum* infections typically show a patchy distribution pattern with hyperendemic foci alternating with low-prevalence or infection-free regions. The life cycle of *A. vasorum* obligatory includes gastropods as intermediate hosts. In 2018, we identified a hyperendemic focus with high gastropod *A. vasorum* prevalences in southern Germany (Obrigheim, Federal State of Baden-Wuerttemberg). To assess whether this hot spot indeed is stable and may therefore bear a significant risk of infection for resident dogs, we decided to perform a long-term follow-up study on *A. vasorum* infections in gastropod species in the area.

Objectives

Aim of the current study was to re-test and potentially confirm the hot spot of *A. vasorum* infections in native gastropod population in Obrigheim, and thereby to start a long-term monitoring to deduce eventual infections risks for resident dogs.

Material and methods

In the summer of 2022, 221 gastropods [215 slugs (*Arion* spp.) and 6 snails (*Helix pomatia*)] were manually collected at the hot spot meadow in Obrigheim. Gastropods were artificially digested and microscopically analysed for the presence of metastrongyloid larvae.

Results

We here confirmed a high prevalence for *A. vasorum* infections in gastropods, since 26.3% (58/221) of specimen were found infected. Of note, comparison to data from summer 2018 (13.6 %) revealed an almost doubling of gastropod *A. vasorum* prevalence in 2022. Thus, a stable *A. vasorum* hot spot may indeed be assumed for this specific meadow. Interestingly, hardly any other metastrongyloid infection was detected. Thus, *Crenosoma* spp. larvae were just found in 0.9% (2/221) of gastropods. All lungworm-infected gastropods belonged to the slug species *Arion* sp., whilst all *Helix pomatia* snail samples proved negative for metastrongyloid larvae. Overall, the larval burden varied from 1 to 241 larvae per slug.

Conclusion

Overall, we here confirmed the presence of an *A. vasorum* "hotspot" in gastropods of Obrigheim. As consequence, resident veterinarians were informed on current findings but hardly showed interest in *A. vasorum* infections. Furthermore, an information board was mounted on the meadow allowing interested dog owners to obtain *A. vasorum*-related information and to fill in an online questionnaire via QR-code scanning.

P57

PANX1-dependent human suicidal NETosis is triggered by the zoonotic parasite *Cryptosporidium parvum* under physioxia (5% O2)

H. Stracke[1], S. S. Hasheminasab[1], I. Conejeros[1], Z. Velásquez[1], U. Gärtner[2], F. Kamena[3], F. Wagenlehner[4], A. Taubert[1], C. Hermosilla[1]

[1]Justus-Liebig-Universität Gießen, Institut für Parasitologie, AG Taubert, Gießen, Germany
[2]Justus-Liebig-Universität Gießen, Institut für Anatomie und Zellbiologie, Gießen, Germany
[3]University of Buea, Laboratory for Molecular Parasitology, Department of Microbiology and Parasitology, Buea, Cameroon
[4]Universitätsklinikum Gießen und Marburg, Urologie, Kinderurologie und Andrologie, Gießen, Germany

Introduction

Cryptosporidium parvum is a zoonotic apicomplexan protozoan parasite causing cryptosporidiosis, a severe enteritis resulting in catharralic diarrhoea, especially in new-borns, toddlers, immunosuppressed patients and calves. Cryptosporidiosis leads to increased toddler mortality worldwide and significant economic losses in the livestock industry. Neutrophil extracellular trap (NET) formation is an important effector mechanism of polymorphonuclear neutrophils (PMN) to fight against viruses, bacteria, fungi and protozoa.

Objectives

The aim was to study the role of distinct signalling pathways (i. e. NF-ƙB, HIF- α, PI-3 kinase), ATP purinergic receptors (PANX1, PX2X1), lactate transporters (MCT1, MCT2) and general metabolic requirements in *C. parvum*-triggered human NETosis.

Material and Methods

Human PMN were isolated from whole blood by negative immunoselection. *C. parvum*-PMN interactions were documented via microscopy, live cell imaging and metabolic assays. For the latter, oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) of PMN were assessed via Seahorse instrumentation. The role of selected signalling pathways was studied via chemical inhibition using CBX, NF499, YC-1, LY-294002, Bay11-7821, AR-C14990 and AR-C155858 to inhibit PANX1, P2X1, HIF1, PI3-kinase, NF-κB, MCT1 and MCT1/MCT2, respectively.

Results

Upon parasite exposure to PMN, immunofluorescence- and confocal microscopy confirmed the presence of classical NET markers (histones and neutrophil elastase) in DNA-positive NET-like structures. Via live cell 3D-holotomographic microscopy, early morphological changes during the NETotic process such as cell membrane extrusions/protrusions and nuclear area expansion (NAE) was documented. *C. parvum*-induced human suicidal NETosis was significantly diminished by PMN pre-treatments with PANX1 inhibitor carbenoxolone (CBX), whilst chemical inhibition of MCT1-, MCT2, HIF-α-, NF-ƙB- and PI-3 kinase failed to affect NETosis under physioxia (5% O2). Overall, neither OCR nor ECAR were changed in *C. parvum*-exposed PMN even though PMN reacted by NET formation.

Conclusion

C. parvum oocysts and sporozoites both induced human suicidal NETosis. We here provide first evidence on a pivotal role of PANX1 in this neutrophil effector mechanism.

P58

Establishment of a commercial Multiplex-Real-time-PCR for the diagnosis of helminth infections

J. Schmiedel[1], *C. Imirzalioglu*[1]

[1]Justus-Liebig-Universität Gießen, Institut für Medizinische Mikrobiologie, Gießen, Germany

Introduction

Helminth infections are a major health burden worldwide which primarily affects subtropical and tropical regions. However, patients originating outside from Europe can import these diseases to Germany. Since our clinic has an infectious diseases department, many patients suspected of having a parasitological infection are treated here.

Objectives

Investigation of stool samples by traditional microbiological methods tends to be rather time and resource consuming. The diagnostic problem increases when it comes to testing procedures for parasitic infections. As in many other laboratories, most of our microbiological stool diagnostics are carried out by syndromic PCR panels. To expand our diagnostic range to parasitic infections, we implemented a Multiplex-Real-time-PCR panel for common helminth infections.

"Patients & methods"

Over a four month period 160 stool samples were screened with the Seegene Allplex™GI-Helminth(I) Assay for presence of gastrointestinal parasites. This assay can detect nine different gastrointestinal parasites. The samples mainly originated from pediatric patients and patients from the infectious diseases department.

Results

In the investigation period an average of 4 samples per week was examined for the presence of parasitic DNA. Parasitic DNA was detected in 9,4% (n = 15) of all tested samples. Positive samples originated from 12 patients. One patient was tested multiple times which accounts for 4 samples. All other patients were sampled only once. Overall, DNA from *Strongyloides* spp. was most frequently detected (n = 6). It was also the only DNA that was detected in addition to DNA from other gastrointestinal parasites twice. The second most common detected parasitic DNA was from *Enterobius vermicularis* (n = 2). DNA from *Necator americanus*, *Ancylostoma* spp., and *Hymnolepsis* spp. was only ever detected once. DNA from *Taenia* sp. *Ascaris* sp. and *Trichuris trichuria* was not found at all.

Conclusion

The Assay could make a useful contribution to the diagnosis of common helminth infections. Nevertheless, it is difficult to make a statement about the amount of parasites, as there is no correlation given between the amount of DNA and the amount of eggs, which is a known problem of Real-time-PCR targeting helminths. In the future, it is planned to use traditional methods to re-examine positive samples. The results should always be discussed with the attending physician in order to include any existing symptoms.

P59

The role of TRP1 in *Plasmodium* sporozoite exit, migration and entry

M. Roy Chowdhury[1], *F. Frischknecht*[1]

[1]Center for Integrative Infectious Disease Research, Uni Heidelberg, Parasitology, Heidelberg, Germany

Introduction

Sporozoites are the transmissible form of the malaria parasite Plasmodium. Sporozoites develop within oocysts in the mosquito midgut of Anopheles mosquitoes. To complete their life cycle they need to exit from the oocysts, invade the mosquito salivary gland, migrate in the skin and enter the liver. The molecular mechanisms of how the sporozoites exit the oocyst and efficiently enter the salivary gland are not clearly understood. TRP1 has been shown to have an important role in activating sporozoite motility within the oocyst and is crucial for its exit from the oocyst. Further experiments show that the C terminus of the protein plays a key role in the egress process and also affects salivary gland invasion.

Objectives

We aim to understand the role of the C terminus of TRP1 in more depth.

Materials & methods

Various molecular genetics methods were used to create different mutations in the C terminus. TRP1 was tagged with GFP to visualize the localization of the protein through live cell imaging and IFA. In vitro gliding assays were done on the sporozoites to assess their motility.

Results & conclusion

This showed that different parts of the TRP1 C-terminus play distinct roles in sporozoite egress, motility and invasion.

P60

Comparison of prototype ELISAs for serodiagnosis of visceral leishmaniasis

B. Menge[1], T. Kruse[1], C. Messing[1], S. Saschenbrecker[1], R. Brückmann[1], A. Gasser[1]

[1]Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany

Introduction

Leishmaniasis is a (sub-)tropical disease that is caused by *Leishmania* protozoa and transmitted by infected sandflies. Diagnosis of visceral leishmaniasis (VL) requires demonstration of *Leishmania sp*. in biopsy material using microscopy, culture, or PCR, which are specific but lack sensitivity in cases with low parasite density. As a non-invasive alternative, serological assays enable examination of the immune response to *Leishmania*.

Objective

Comparing the performance of three prototype ELISAs for the detection of *Leishmania*-specific antibodies.

Materials & methods

The prototype ELISAs were based on three substrates: i) *Leishmania donovani* lysate, ii) *Leishmania donovani* excretory/secretory (E/S) proteins from cell culture supernatant, iii) recombinant K39. Assay performance was compared between these prototypes as well as with established anti-*Leishmania* ELISAs (Bordier, Virion\Serion, Vircell) using serum samples from 52 VL patients and 75 healthy controls (25 blood donors, 25 pregnant women, 25 children). Additionally, a cross-reactivity panel was tested, comprising 33 serologically pre-characterized samples from patients with other parasitic infections (15 anti-*Trypanosoma cruzi* IgG positive, 8 anti-*Schistosoma mansoni* IgG positive, 10 anti-*Plasmodium* IgG positive).

Results

Among the prototype ELISAs, the best discrimination between VL patients and healthy controls was observed with the E/S-based ELISA, yielding a sensitivity of 90% (IgG) to 96% (IgG/IgM) at a specificity of 100%, with cross-reactivity rates between 0% for samples positive for *Schistosoma* or *Plasmodium* and 100% for *Trypanosoma-positive samples*. The K39-based ELISA performed with a sensitivity of only 58% (IgG, IgG/IgM), while specificity and cross-reactivity amounted to 100% and 0%, respectively. In contrast, the established ELISAs showed sensitivities between 86% (Bordier, IgG) and 94% (Vircell, IgG/IgM) [specificity data not available], with cross-reactivity ranging from 0% (*Schistosoma, Plasmodium*) to 100% (*Trypanosoma*).

Conclusion

The use of *Leishmania donovani* E/S antigen provides highest ELISA sensitivity and specificity for the detection of VL-associated antibodies but implies substantial cross-reactivity with *Trypanosoma*. The K39 antigen, however, results in a highly specific ELISA that is devoid of cross-reactivity. Future studies will aim at optimizing the antigenic substrates to enhance the serodiagnostic performance for VL.

P62

Application of Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry for identification of adult tapeworms: *Moniezia* spp. and *Thysaniezia* spp.

L. Conrad[1], E. O. Ngbede[1,2,3], M. I. Adah[2], S. T. Yusuf[4], S. L. Becker[1,5,6], I. Sy[1]

[1]Institute of Medical Microbiology and Hygiene, Saarland University, Homburg, Germany
[2]College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria
[3]German Collection of Microorganisms and Cell Culture, Leibniz Institute DSMZ, Braunschweig, Germany
[4]Kubwa Abattoir, Public Health Section, Department of Veterinary Services, Federal Capital Territory Administration, Abuja, Nigeria
[5]Swiss Tropical and Public Health Institute, Basel, Switzerland
[6]University of Basel, Basel, Switzerland

Introduction

Moniezia spp. and *Thysaniezia* spp. are intestinal parasites of ruminants that infest the small intestine. Infection with tapeworms occurs worldwide and can lead to symptoms such as diarrhoea, anaemia, and even death in ruminants. The diagnosis of cestodes is mainly based on microscopic observations of eggs and/or molecular detection. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is employed in routine laboratories for the identification of bacteria, fungi, and mycobacteria. Recent studies showed that this method can also be applied for helminths identification.

Objectives

The aim of this study was to assess the capacity of MALDI-TOF MS as a new alternative tool for the identification of adult tapeworms from cattle: *Moniezia* and *Thysaniezia* species.

Material and Methods

Sixty-three tapeworms collected from the intestine of slaughtered cattle in Nigeria were morphologically identified as *Moniezia* spp. and *Thysaniezia* spp. Molecular confirmation was performed on 9 randomly selected samples to serve as a reference for further MALDI-TOF MS analysis. For this, they were subjected to a protein extraction protocol and measured by MALDI-TOF MS. The obtained spectra were used to create so-called main specific spectra (MSPs) and added to the in-house database. To validate the newly created in-house database, the remaining samples were tested with it after long storage in ethanol. A Log score value (LSV) higher than 1.7 was considered a reliable identification. Furthermore, a clustering analysis (dendrogram) was realized to evaluate the relatedness of the different species.

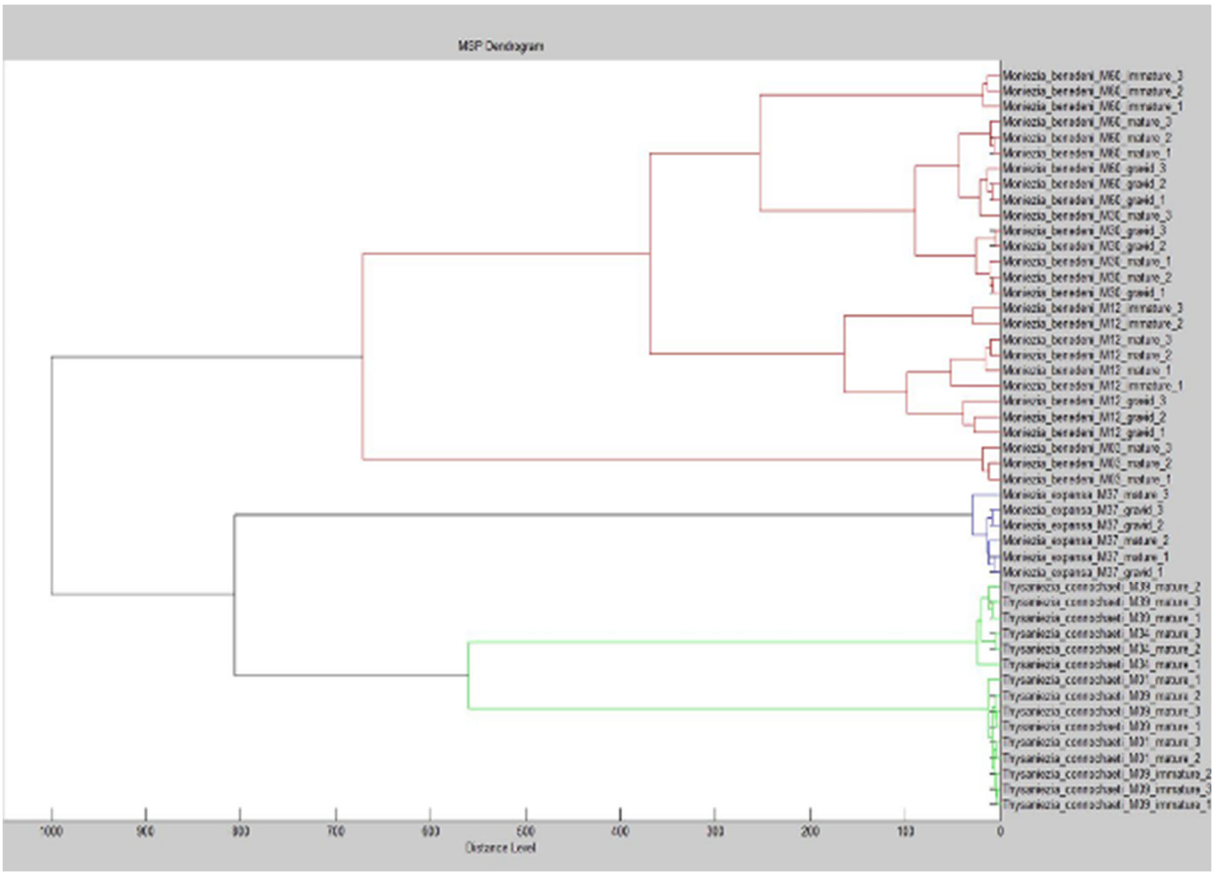
Results

The 9 adult tapeworms were molecularly confirmed with the detection of the following species: *Moniezia benedeni, Moniezia expansa*, and *Thysaniezia connochaeti*. When the in-house database implemented with those species was subsequently tested with the remaining 54 adult tapeworms after long storage in ethanol, 55.1 % of correct identification with an average LSV above 1.7 was achieved. The dendrogram analysis showed a clear separation between the different species (Figure 1).

Conclusion

We conclude that MALDI-TOF MS can be applied for the identification of cestodes. Nevertheless, future research is needed to improve the process.

Fig. 1


MSP Dendrogram
Moniezia_beneden_M60_immature_3
Moniezia_beneden_M60_immature_2
Moniezia_beneden_M60_immature_1
Moniezia_beneden_M60_mature_3
Moniezia_beneden_M60_mature_2
Moniezia_beneden_M60_mature_1
Moniezia_beneden_M60_gravid_3
Moniezia_beneden_M60_gravid_2
Moniezia_beneden_M60_gravid_1
Moniezia_beneden_M08_mature_3
Moniezia_beneden_M08_gravid_3
Moniezia_beneden_M08_gravid_2
Moniezia_beneden_M08_mature_1
Moniezia_beneden_M08_mature_2
Moniezia_beneden_M08_gravid_1
Moniezia_beneden_M12_immature_3
Moniezia_beneden_M12_immature_2
Moniezia_beneden_M12_mature_3
Moniezia_beneden_M12_mature_2
Moniezia_beneden_M12_mature_1
Moniezia_beneden_M12_immature_1
Moniezia_beneden_M12_gravid_3
Moniezia_beneden_M12_gravid_2
Moniezia_beneden_M12_gravid_1
Moniezia_beneden_M03_mature_3
Moniezia_beneden_M03_mature_2
Moniezia_beneden_M03_mature_1
Moniezia_expansa_M37_mature_3
Moniezia_expansa_M37_gravid_3
Moniezia_expansa_M37_gravid_2
Moniezia_expansa_M37_mature_2
Moniezia_expansa_M37_mature_1
Moniezia_expansa_M37_gravid_1
Thysaniezia_ceenochaeti_M39_mature_2
Thysaniezia_ceenochaeti_M39_mature_3
Thysaniezia_ceenochaeti_M39_mature_1
Thysaniezia_ceenochaeti_M34_mature_3
Thysaniezia_ceenochaeti_M34_mature_2
Thysaniezia_ceenochaeti_M34_mature_1
Thysaniezia_ceenochaeti_M31_mature_1
Thysaniezia_ceenochaeti_M39_mature_2
Thysaniezia_ceenochaeti_M39_mature_3
Thysaniezia_ceenochaeti_M39_mature_1
Thysaniezia_ceenochaeti_M31_mature_3
Thysaniezia_ceenochaeti_M31_mature_2
Thysaniezia_ceenochaeti_M39_immature_2
Thysaniezia_ceenochaeti_M39_immature_3
Thysaniezia_ceenochaeti_M39_immature_1
1000
900
800
700
600
500
400
300
200
100
0
Distance Level

P63

Prevalence of malaria and typhoid co-infection among out patients attending Ijanikin health center, Ijanikin, Lagos state, Nigeria

J. Ajayi[1,2], *A. Ajayi*[1,2]

[1]Ogun State Institute of Technology, Igbesa, Ogun State, Microbiology, Nigeria, Nigeria
[2]Lagos State University, Science Education, Lagos, Nigeria

PREVALENCE OF MALARIA AND TYPHOID CO-INFECTION AMONG OUT PATIENTS ATTENDING IJANIKIN HEALTH CENTER, IJANIKIN, LAGOS STATE, NIGERIA

Ajayi J. B1 and Ajayi O. A2

Department of Science Laboratory Technology, Microbiology Unit, Igbesa and Araromi Ilogbo Senior Secondary School, Oko Afo, Lagos.

Background : Malaria and salmonella infections are endemic especially in developing countries; however malaria and salmonella co-infection is a rare entity with high mortality. An association between malaria and typhoid fever was first described in 1862 in North America as an entity called typho-malaria fever (Smith, 1982) I**ntroduction:** .A study was carried out on patients clinically diagnosed of malaria and typhoid at Otto Ijanikin health center, Lagos State, Nigeria to verify the degree of relationship between malaria and typhoid fever. **Methods:** A total number of 200 patients were sampled. Widal kit and Rapid diagnostic were used for typhoid and malaria diagnoses respectively. Plasmodium falciparum was the only parasite used to indicate the presence of malaria in the patients. **Result**: The study indicated that out of 200 patients, 50 (25%) were positive for malaria of which 16(32%) were male while 34 (68%) were female. A total number of 69 patients were positive for typhoid of which 24 were male and 45 were female. Out of 16 male with malaria, 10(62.5%) were typhoid positive and out of 34 female positive for malaria, 18(52.9%) were positive for typhoid infection. **There is no positive relationship between malaria and typhoid (**$p < .05$). The results also indicate that the differences in plasmodium falciparum parasiteamia and the antibody titre of Salmonnela is significant in using the widal test**.** Conclusion**: Using only the widal test to diagnose typhoid fever infection for patients that are positive for malaria should be discouraged..**

P64

IgE-based diagnosis using transgenic reporters: Game changer in *Echinococcus* sp. diagnosis?

S. Shabir[1], *F. H. Falcone*[1]

[1]Institute of Parasitology, Gießen, Germany

Introduction: Despite its importance as a widespread public health threat, *Echinococcus* sp. is one of the 20 Neglected Tropical Diseases (NTDs) targeted by the WHO for control and eradication. Echinococcosis has a global economic impact on both human and livestock health, as well as food security. The gold standard for echinococcosis diagnosis is the use of imaging technologies such as CAT, MRI or Ultrasonography. However, such methods are not widely available in endemic countries. Therefore, cheaper test such as serology would be an advantage. However, such tests, usually based on IgG detection, are unreliable due to low specificity. Despite the central role of IgE responses in metazoan parasite infections, this isotype has not been used for diagnostic purposes of Echinococcosis. Therefore, we are developing a highly sensitive and specific serological assay based on humanised and caninised rat basophil leukaemia (RBL) IgE reporter cell lines.

Materials and methods: We nucleofected RBL-NPY-mRFP cells with 3 constructs, expressing the wild type dog FceR1a, a chimeric Dog/Rat FcεRIα and a wild type dog FcεRIα chain in which three potential endoplasmic reticulum retention signals have been removed.

Results: Successful transfection of these cells with the 3 constructs was verified by PCR and in the next step its functionality will be tested through functional assays. The second part of the project involves the choice and recombinant expression of suitable diagnostic antigens. As the reporter assay relies on detection of antigen-specific IgE, we need to select parasitic antigens that are the target of an IgE response (i.e. allergens). After a bioinformatics assessment of predicted allergens of *Echinococcus granulosus* and synthesis of the corresponding cDNA, we are expressing these antigens/allergens using HEK2936E cells grown in suspension.

Conclusion: Once developed, the RBL-NPY-mRFP expressing dog FcεRIα will be assessed for its performance and compared to available serological techniques.

P65

Development of a Fluorescent RBL Reporter System for Diagnosis of Porcine Cysticercosis

M. S. Hossain[1], P. Toye[2], L. F. Thomas[2], F. H. Falcone[1]

[1]Justus-Liebig-Universität Gießen, Institute for Parasitology, Gießen, Germany
[2]International Livestock Research Institute (ILRI), Nairobi, Kenya

Introduction:

Porcine cysticercosis (PCC) is a World Organization for Animal Health listed notifiable disease, caused by the larval stage of *Taenia solium.* Pigs get infected by ingesting human stool or water/vegetation contaminated with *T. solium* eggs. The disease is endemic in Latin America, sub-Saharan Africa, South and South East Asia. PCC hampers food security and affects livelihoods of pig farmers resulting in reduced pork value and economic loss, especially in developing countries. Available serological diagnostic tests based on IgG detection are characterised by low specificity.

Objectives:

This study aims to assess suitability of detecting parasite-specific Immunoglobulin E (IgE) using species-specific IgE reporter cell lines. Our objectives are to create a reporter cell line that is able to bind pig IgE and to identify, clone, and recombinantly express candidate *T. solium* antigens, which will be assessed for their suitability as "diagnostic allergens" for diagnosis of PCC.

Materials and methods:

We used pcDNA5 as vector backbone for cloning of a synthetic pig high affinity IgE receptor alpha chain (SsFCERIA). After ligation and bacterial transformation, we transfected our target transformant (SsFCERIA/pcDNA5) into RBL NPY-mRFP reporter system to develop "porcinised" reporter system (RBL NPY-mRFP SsFCERIA) and for further selection of stable transfectant cell lines. We selected 10 predicted allergens of *T. solium* (Q9NI46, Q2XNL7, K0A0S9, E5LBB8, A3F4Q9, A3F4W9, A3F4U5, A3F4Y2, A3F4V0, and A3F4Y4) for further bioinformatic analysis.

Results:

From bioinformatic analysis, we found a signal peptide in one allergen, N-linked glycosylation site in 4, and different protein motifs (e.g. FABP, EF-Hand 1, and Trypsin-like), but no transmembrane regions. The corresponding cDNAs will be used for recombinant expression using HEK293-6E cells grown in suspension.

Conclusion:

Our study will enable development of a novel diagnostic tool to improve the standard of current serological diagnosis of PCC, which may help to interrupt zoonotic transmission of *T. solium* in endemic countries.

ISBN: 978-3-948023-49-2