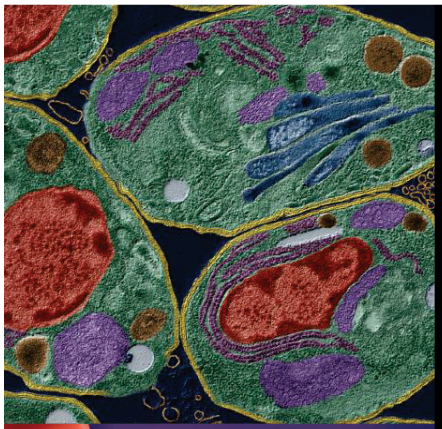
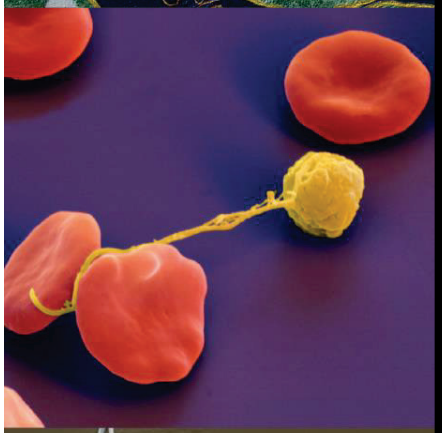




GRK 2046 "Parasite Infections: From Experimental Models to Natural Systems"

Annual Retreat 2018

Veterinarium Progressum

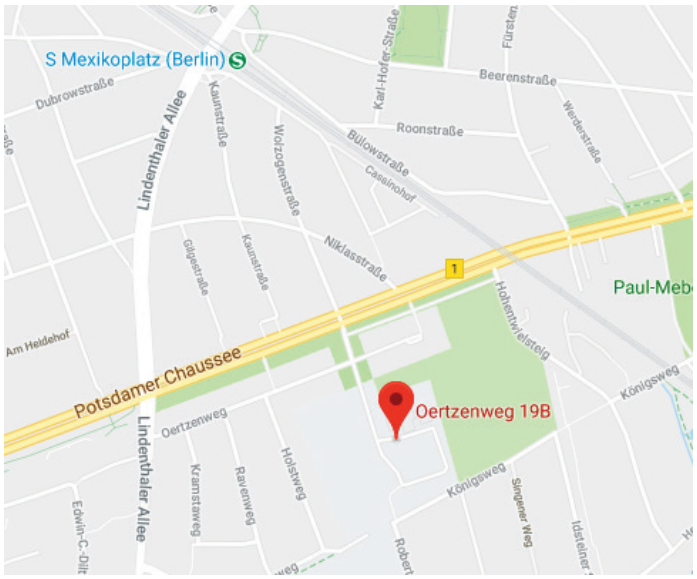
VetMed faculty

29th and 30th October 2018

Notes:

Location:

Veterinarium Progressum
VetMed faculty
Oertzenweg 19B, 14163 Berlin



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Irina Diekmann: irina.diekmann@fu-berlin.de

Lubomír Bednář: bednarlu@hu-berlin.de

Program

29th October (Monday)

09:00	**Welcome Address**
09:15 - 09:50	**Talk Session 1 - *Wildlife***
09:50 - 10:15	Coffee break
10:15 - 11:00	**Talk Session 2 - *Wildlife***
11:00 - 11:15	Short break
11:15 - 12:15	**Talk Session 3 - *Helminths***
12:15 - 13:15	Lunch
13:15 - 13:50	**Talk Session 4 - *Helminths and Toxoplasma***
13:50 - 14:00	Short break
14:00 - 14:45	**Talk Session 5 - *Helminths and Toxoplasma***
14:45 - 15:15	Coffee break
15:15 - 16:00	**Talk Session 6 -*Toxoplasma***
16:15 - 17:00	**Principal Investigator Meeting and Doctoral Researcher Meeting**
17:00 - 19:00	**Quiz, Games, Snacks**
19.30 open end	**Social evening** Dinner: Peter Pane, Schloßstraße 34, 12163 Berlin

Program

30th October (Tuesday)

09:00	**Welcome**
09:15 - 10:00	**Talk Session 1 - *Giardia***
10:00 - 10:15	Short break
10:15 - 11:00	**Talk Session 2 -*Plasmodium***
11:00 - 11:30	Coffee break
11:30 - 12:15	**Talk Session 3 - *Plasmodium***
12:15 - 13:15	Lunch
13:15 - 13:55	**Talk Session 4 -*Mosquitoes***
13:55 - 14:00	**Closing Speech**
14:15 - 15:00	**Doctoral Researchers, Speaker and Coordinator Meeting**

Day 1: TALK SESSION OVERVIEW

Welcome Address
Speaker GRK 2046: Susanne Hartmann
Monday 09:00 – 09:15

Talk Session 1: *Wildlife*
Monday 09:15 – 09:50

Moderators: Alexander Gerhard and Robin Benter

Name	Title	Abstract page
Susana Ferreira	Intrinsic and extrinsic determinants of helminth parasite infection in female spotted hyenas	13
Miguel Veiga	Fitness consequences and determinants of parasite infections in a wild social carnivore, the spotted hyena (*Crocuta crocuta*)	14
Maria Serocki	Apicomplexan parasites, immunity and its link to life history states in free-ranging cheetahs	15

Talk Session 2: *Wildlife*
Monday 10:15 – 11:00

Moderators: Totta Ehret and Bhavya Kapse

Name	Title	Abstract page
Lubomir Bednar	Immunological characterization of Hybrid Vigor phenomenon in *Mus musculus* subspecies during Eimeria infections	16
Victor Hugo Jarquin-Diaz	Phylogenetic diversity of *Eimeria* spp. natural populations of the house mouse (*Mus musculus*) across the European Hybrid Zone	17
Vivian Schüler	tba	

Talk Session 3: *Helminths*
Monday 11:15 – 12:15

Moderators: Susana Ferreira and Martin Kraft

Talk Session 4: *Helminths and Toxoplasma*
Monday 13:15 – 13:50

Moderators: Esra Yilmaz and Maria Serocki

Talk Session 5: *Helminths and Toxoplasma*
Monday 14:00 – 14:45

Moderators: Florence Awamu and Costanza Tacoli

Talk Session 6: *Toxoplasma*
Monday 15:15 – 16:00

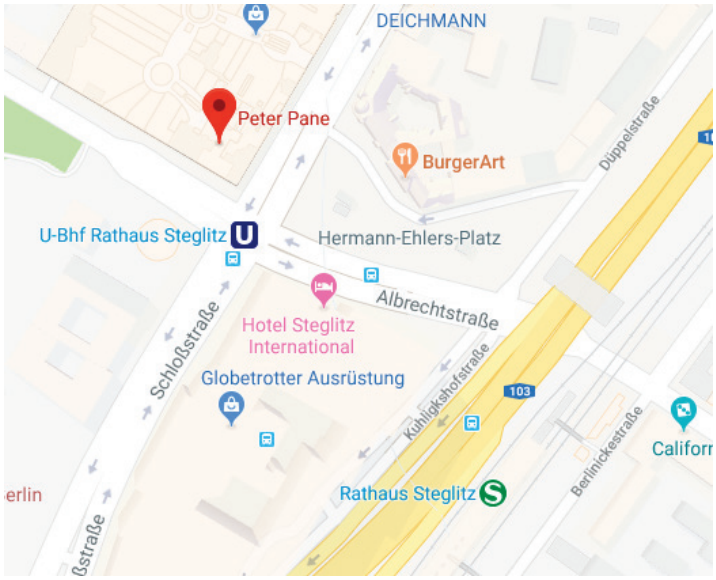
Moderators: Ivet Yordanova and Welmoed van Loon

Name	Title	Abstract page
Francesca Torelli	Identifying cat transmission-relevant rodent reservoirs for virulent *Toxoplasma gondii* strains by analyzing IRGs-mediated resistance mechanisms	28
Estefania Delgado-Betancourt	Innate immune responses of intestinal organoids from wild rodents upon *Toxoplasma gondii* infection	29
Benedikt Fabian	Oocyst-molecules of *Toxoplasma gondii*: Their role for survival in the environment and their diagnostic potential	30

Principal Investigator Meeting (1st floor Veterinarium Progressum)
Doctoral Researcher Meeting (Ground floor Veterinarium Progressum)
Monday 16:15 – 17:00

Quiz, Games, Snacks (Veterinarium Progressum)
Monday 17:00 – 19:00

Social evening: Dinner at Peter Pane (Schloßstraße 34, 12163 Berlin)
Monday 19:30 – open end



Day 2: TALK SESSION OVERVIEW

Talk Session 1: *Giardia*
Tuesday: 09:15 – 10:00

Moderators: Francesca Torelli and Caroline Kiuru

Name	Title	Abstract page
Ivet Yordanova	Shifts in Treg/Th17 balance correlate with differential control of infection with *Giardia muris*	31
Totta Ehret	Influences of metabolic stress on *Giardia duodenalis* growth	32
Martin Kraft	Human small intestinal organoids - a superior model to investigate *Giardia* sp. infection	33

Talk Session 2: *Plasmodium*
Tuesday: 10:15 – 11:00

Moderators: Nicole Affinass and Benedikt Fabian

Name	Title	Abstract page
Jonnel Jaurigue	Characterisation of antibody responses against GPI-epitopes	34
Costanza Tacoli	Characterization of *Plasmodium falciparum* artemisinin resistance in southern Rwanda, 2010-2018	35
Welmoed van Loon	Association between α-thalassemia and artemisinin effectivity on *Plasmodium falciparum in vitro* and in the field	36

Talk Session 3: *Plasmodium*
Tuesday: 11:30 – 12:15

Moderators: Norus Ahmed and Benjamin Hamid

Name	Title	Abstract page
Oriana Kreutzfeld	Pre-clinical evaluation of a triple genetically arrested parasite (tKO GAP) for malaria vaccine development	37
Calvin Yan Ki Hon	Expression of *Salmonella enterica* flagellin in transgenic *Plasmodium berghei* as adjuvant to potentially improve malarial vaccine efficacy	38
Florence Awamu	The role of *Plasmodium falciparum* derived microvesicles in malaria related anemia	39

Talk Session 4: *Mosquitoes*
Tuesday: 13:15 – 13:55

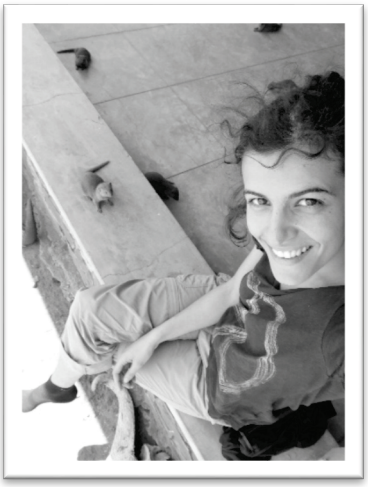
Moderators: Ankur Midha and Calvin Yan Ki Hon

Name	Title	Abstract page
Suzana Zakovic	Role of REL2/IMD pathway in the vectorial capacity of malaria mosquito *Anopheles gambiae*	40
Robin Benter	The role of microbial communities in structuring mosquito populations in Mali	41
Caroline Kiuru	Flourishing in germs: Deciphering the role of bacteria in development of the malaria vector *Anopheles gambiae*	42
	Welcome new Students	43

Closing Speech
Tuesday: 13:55 – 14:00

Doctoral researchers, Speaker and Coordinator Meeting (Veterinarium Progressum)
Tuesday: 14:15 – 15:00

Intrinsic and extrinsic determinants of helminth parasite infection in female spotted hyenas



Susana Ferreira

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C04 (Gen 1)

There are substantial differences in parasite composition and infection load between individuals in wildlife populations. Knowledge of factors determining these differences and their impact on Darwinian fitness is limited. Laboratory studies have uncovered several mechanisms that modulate parasite infection, including exposure to parasite infective stages, immunological responses and their downregulation by resource allocation trade-offs, and parasite manipulation of the host's immune system. This project aims i) to investigate determinants shaping gastrointestinal parasite load in spotted hyenas in three clans in the Serengeti National Park, Tanzania, and ii) to determine the fitness consequences of infection. The intestinal biome of spotted hyenas in the Serengeti National Park was analysed using a multi-amplicon sequence approach and classical coprological methods and several determinants onparasite infection were investigated, including rank, age, co-infections, clan composition and clan identity. Furthermore, our results indicated that an energetically costly parasite load with the helminth *Ancylostoma* decreases juvenile survival in spotted hyenas and that the effect of social status on survival is modulated by age. These findings reveal that heterogeneity of parasite infections in a wild mammal population is shaped by a complex range of factors and helminth infections have long term consequences.

Publications:

1. Heitlinger E*, **Ferreira SCM***, Thierer D, Hofer H, East ML. (2017). The Intestinal Eukaryotic and Bacterial Biome of Spotted Hyenas: The Impact of Social Status and Age on Diversity and Composition. *Front Cell Infect Microbiol 7*:262. doi: 10.3389/fcimb.2017.00262. *Equal contribution
2. Selmann, A., Webster, F., **Martins Ferreira**, S., Czirják, G., Wachter, B. 2018. Age-specific gastrointestinal parasite shedding in free-ranging cheetahs (Acinonyx jubatus) on Namibian farmland. In rev. Int J Parasitol Parasites Wildl. doi: 10.3389/fcimb.2017.00262
3. **Ferreira S.C.M.** *, Torelli F. *, Klein S., Fyumagwa R., Hofer H., Seeber F., East M.L. Seroprevalence of Toxoplasma gondii in African carnivores in East Africa. Int J Parasitol Parasites Wildl (**in revision**) (2018) (* shared first authorship)
4. **Martins Ferreira**, S., Braun, B., Hofer, H., East, M.L. Czirják, G. 2018. Development and establishment of non-invasive immune methods in spotted hyenas. **In prep**.
5. **Martins Ferreira,** S.*, Balard, A.*, Hofer, H., Heitlinger, E., East, M.L. 2019. Non-invasive immune gene expression in a non-model animal - the spotted hyena. **In prep**. * shared first authorship

Fitness consequences and determinants of parasite infections in a wild social carnivore, the spotted hyena *(Crocuta crocuta)*

Miguel Veiga

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Leibniz-Institut für Zoo- und Wildtierforschung (IZW) Berlin

C04 (Gen 2)

Currently, relatively little is known about the Darwinian fitness consequences that parasite infections have on their wild mammalian hosts because of the extreme complexity of parasite-host interactions in natural systems. Parasite transmission and host infection depends on many factors such as parasite genotype, life-cycle, host(s) density and life history states, and environmental factors that affect the survival of the infective stages of parasites. Natural host populations often have a high level of heterogeneity between individuals in parasite infection load and the occurrence of co-infections that are thought to result from complex interactions between intrinsic factors, such as life history stage, nutritional status and host immune genotype, and extrinsic factors such as environmental factors, including the social environment in group living mammals. Our research aims to investigate the effect of energetically costly intestinal parasites (*Ancylostoma* and *Cystoisospora*) on host immune function, in terms of host immune genotype and immune processes. The proxy measures of Darwinian fitness we will use include survival, reproductive success and longevity. Our host species is the spotted hyena (*Crocuta crocuta*): a carnivore that lives in clans with linear social dominance hierarchies. The study population consists of several hundred, individually known free-ranging animals in the Serengeti National Park, Tanzania. Long-term detailed monitoring of this study population will provide information on life history, social rank and a wide range of other factors. In order to investigate the link between parasite infection and host immune processes and their effect on fitness during various life stages throughout an animal's life-span, I will apply non-invasive methods to conduct a multifaceted study using longitudinal life-histories and relevant biological samples obtained throughout the life span of known individuals. The first aim is to apply non-invasive immunological assays, developed by the 1st generation GRK PhD student, Susana Ferreira, to investigate the relationship between faecal parasite egg/oocyst load, immune responses and proxy fitness measures. We validated assays for faecal samples which include methods for 1) faecal O-linked oligosaccharides (mucins); 2) total faecal IgA and IgG levels; 3) faecal lysozyme concentrations and 4) faecal cytokine expressions. We further plan to develop faecal inflammatory (e.g. haptoglobin, neopterin) markers. Integrating these parameters will hopefully provide insights on their role in mucosal immunity. Furthermore, we aim to extend the knowledge on spotted hyena innate immune genes, building on previous work on Toll-like receptors. We hypothesize that parasite-driven selection should modulate the allele composition for immune genes, and besides describing diversity in functionally important antigen-binding sites we would like to integrate these results with cytokine expression levels and Darwinian fitness measures such as longevity. This project will combine concepts from life history theory, immunology and genetics, ultimately aiming for a comprehensive interpretation of factors modulating heterogeneity of infection across individuals in wildlife populations.

Apicomplexan parasites, immunity and its link to life history states in free-ranging cheetahs

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C01 (Gen 2)

Free-ranging cheetah populations are threatened, thus it is important to investigate their health status and potential infection risks. Cheetahs have a low genetic variability, including at the major histocompatibility complex (MHC), which is an important gene group involved in adaptive immune responses. Therefore, cheetahs might be particularly vulnerable to parasites. Nevertheless, free-ranging cheetahs do not show any pathological or clinical evidence for diseases, although animals come into contact with numerous macro- and microparasites and seroconvert when exposed to them. This study aims at investigating the effect of the low number of MHC alleles in cheetahs on its immune system and hemoparasite infections. For this purpose, I will examine (1) MHC dependent and independent cellular immune components, (2) immune responses depending on life history states, (3) co-infection of different hemoparasites and (4) parasite prevalence and diversity depending on life history states. The results of the immune profiles and infection status of free-ranging cheetahs will be compared with sympatric living free-ranging leopards, a species with a high MHC diversity. Preliminary results demonstrate that cheetahs are highly co-infected with helminths, bacteria and apicomplexan parasites despite their healthy appearance. These and prospective results will help to understand the dynamics of immunity and parasite susceptibility in a threatened carnivore species and thereby improve the success of conservation management strategies.

Immunological characterization of Hybrid Vigor phenomenon in *Mus musculus* subspecies during Eimeria infections

Lubomír Bednář

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Supervisor: **Emanuel Heitlinger**

Humboldt-Universität zu Berlin

new C08 (Gen 2)

The fields of current evolutionary ecology and molecular immunology are vastly different due to their wide scopes of investigation, and rarely coincide. In the study presented here, we aim to investigate a phenomenon known as Hybrid Vigor observed in the European House Mouse Zone, dissect its immunological mechanism and provide answers via addressing the key aspects forming this unique interplay of immunology and evolutionary ecology. The aforementioned hybrid zone can be found spanning through Northern, Central and Southern Europe, where the Western house mice (*Mus musculus domesticus*) and Eastern house mice (*Mus musculus musculus*) meet, procreate and generate populations of hybrid mice which under normal conditions do not establish as separate species due to reduced fitness. However, it has been indicated in previous research (Alice Balard, Víctor Hugo Jarquín Díaz), that the hybrid mice perform better than their wild derived inbred subspecies cousins (Totta Ehret, Francisca Böhning), and their wild derived outbred subspecies cousins (Alice Balard, ,Víctor Hugo Jarquín Díaz, Enas Khalifeh), when infected by intracellular parasites such as *Eimeria*. My current project therefore focuses on collecting samples from wild and wild-derived mice of both mouse subspecies and their hybridized offspring, under the condition of *Eimeria falciformis* and *Eimeria ferrisi* infections. The project will further continue into establishing immunological profiles (mouse strains vs. parasite strains), examining cross-infection scenarios, wild to wild-derived immunological profile comparative study and immune cell population counts.

Phylogenetic diversity of *Eimeria* spp. natural populations of the house mouse (*Mus musculus*) across the European Hybrid Zone

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new C08 (Associated)

Eimeria is described as an intracellular and host specific parasite; however in the case of species that infect rodents their classification is not clear because it has been shown that some species do not fit with the host specificity pattern. In this work, we discuss if this group of coccidians are misclassified by the currently molecular markers or if these species have a broad host range. Molecular identification, morphological description and tissue localization of *Eimeria* were done for isolates from natural populations of hosts (*Mus musculus domesticus, M. m. musculus* and hybrids). We compared this information to previously reported datasets in rodents from different families. Using this approach, three different species of *Eimeria* were identified infecting house mice, *E. ferrisi*, *E. falciformis* and *E. vermiformis*, being the first one the most prevalent. We also observed that using the most reported genetic markers (COI and 18S rDNA) *E. falciformis* and *E. vermiformis* cluster together with previously reported *E. apionodes* strains identified in different rodent species (*Apodemus* sp.*, Myodes* sp.*,* and *Microtus sp.*) suggesting that these group of coccidians could be forming a species complex with a wide host range infecting murid and cricetid rodents. Nevertheless, we cannot discard the possibility that the genetic markers are not polymorphic enough to differentiate among these organisms. Therefore deeper studies on the current taxonomy using multiple markers and even genome comparisons would be necessary to provide more evidences to differentiate them and complement the current morphological information used to identify these coccidians.

Understanding cytochrome P450-mediated anthelmintic resistance in nematodes

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A05 (Gen 1)

Due to their broad-spectrum activity and affordability, benzimidazole (BZ) and macrocyclic lactone (ML) anthelmintics had revolutionized parasite control in livestock. However, as a result of their excessive use the livestock industry now faces a global problem with anthelmintic resistance. Alterations at the drug target site as well as drug elimination by efflux proteins have been continuously investigated as a cause of resistance. More recently, drug metabolization via cytochrome P450 enzymes (CYPs) are also considered plausible. In the free-living nematode *Caenorhabditis elegans*, CYP35D1 has been shown to metabolize a BZ. Using molecular approaches, the PhD project aimed to understand whether this observation can be extrapolated to *Haemonchus contortus* – a gastro-intestinal parasite of ruminants. In addition, *in vitro* drug assays were used to investigate the role of CYPs in the modulation of ML susceptibility in *C. elegans*. Results of these approaches will be presented and approaches for future studies will be provided.

Publications

1. **Yilmaz, E**, Wongkamchai, S, Ramünke, S, Koutsovoulos, GD, Blaxter, ML, Poppert, S, Schaper, R, von Samson-Himmelstjerna G, Krücken J. 2018. High genetic diversity in the *Dirofilaria repens* species complex revealed by mitochondrial genomes of feline microfilaria samples from Narathiwat, Thailand. *Transboundary and Emerging Diseases,* doi: 10.1111/tbed.13033.
2. **Yilmaz, E**, Ramünke, S, Demeler, J, Krücken, J. 2017. Comparison of constitutive and thiabendazole-induced expression of five cytochrome P450 genes in fourth-stage larvae of *Haemonchus contortus* isolates with different drug susceptibility identifies one gene with high constitutive expression in a multi-resistant isolate. *International Journal for Parasitology: Drugs and Drug Resistance*, 7, 362-369.
3. **Yilmaz, E**, Fritzenwanker, M, Pantchev, N, Lendner, M, Wongkamchai, S, Otranto, D, Kroidl, I, Dennebaum, M, Thanh, H, Tran, A, Ramünke, S, Schaper, R, von Samson-Himmelstjerna, G, Krücken, J. 2016. The mitochondrial genomes of the zoonotic canine filarial parasites Dirofilaria (Nochtiella) repens and Candidatus *Dirofilaria (Nochtiella)* hongkongensis provide evidence for presence of cryptic species. *PLoS Neglected Tropical Diseases,* 10(10): e0005028. doi:10.1371/journal.pntd.0005028
4. **Yilmaz, E**, Kulke D, von Samsons-Himmelstjerna G, Krücken J. 2015. Identification of novel splice variants of the voltage- and Ca(2+)-dependent K(+)-channel SLO-1 of *Trichuris muris*. *Molecular and Biochemical Parasitology,* 199, 5-8.

Identification and characterization of ML metabolic pathways in *Cooperia oncophora*

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A05 (Gen 2)

Drug resistance is an emerging problem in helminth diseases especially caused by trichostrongyloid nematodes. One of the most important parasitic gastrointestinal nematodes is Cooperia oncophora, which is generally treated with broad-spectrum anthelmintics such as benzimidazoles (BZs) or macrocyclic lactones (MLs). MLs bind to specific glutamate-gated chloride channels (GluCl) causing paralysis. Mutations in the extracellular domain of the GluCl channels were associated with resistance. However, these findings were not sufficient to explain resistance in various field-derived isolates of several parasitic nematode species. Therefore, ML resistance is suspected to be multi-genic. Associations between drug resistance and expression of drug biotransformation enzymes as cytochrome P450 (CYP) has been confirmed. Nevertheless, xenobiotic metabolism is catalyzed by a number of different enzyme classes such as FAD-dependent monooxygenases (FMOs), glutathione S- or UDP-glycosyl transferases (GSTs and UGTs). Currently, it is unclear which of these enzyme classes are involved in resistance to the individual drug classes.

This study aims to identify and characterize candidate genes responsible for drug metabolism of *C. oncophora.* Adult worms will be isolated from duodenum of infected calves, *in vitro* cultivated and exposed to a sublethal concentration of different anthelmintics. Subsequently, RNAseq will be used to identify genes with inducible expression after exposure to ivermectin, moxidectin, thiabendazole and levamisole. Identified candidate genes are going to be analyzed by functional analysis focusing on rescue experiments in *Caenorhabditis elegans*. Candidate genes will be integrated to the same genomic locus under control of the gut specific ges-1 promoter. The effects of anthelmintics on the development of transgenic *C. elegans* will be investigated by recording concentration-response-curves.

Cyathostomin population diversity in treated and untreated equine hosts with different geographical background

Irina Diekmann

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Supervisors: **Georg von Samson-Himmelstjerna and Jürgen Krücken**

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C05 (Gen 2)

Cyathostomins currently represent the most important intestinal parasites in horses. Larval cyathostominosis is a threat to equine health and welfare for all grazing horses. As a standard approach for the control of cyathostomin infections and the prevention of cyathostominosis regular anthelmintic treatments are being employed. However, successful treatment is hampered by frequent occurrence of anthelmintic resistance in cyathostomin populations. Currently, 50 described cyathostomin species are considered to be valid and mixed infections with 5-10 but even up to 15 species in one horse are typically observed. To date, little is known about the individual pathogenicity, population biology or AR-characteristics of the cyathostomin species-complex. A major problem in this context is that species-specific identification can only reliably be performed by morphologically determination of adult worms for which just a few researchers worldwide have the required expertise. Therefore, additional and readily transferable tools for the species-identification are urgently needed.

With the help of Next Generation Sequencing, we want to analyse the nemabiome of cyathostomins in order to draw conclusions about population diversity and dynamics within a host. With regard to the current resistance situation, samples from Brazil, Ukraine, Kentucky, Ireland and Germany will be investigated. Per country, 10 regularly treated horses and 10 horses from herds that have not been treated for decades will be compared. From Ukraine, there are additional samples from donkeys, kulans and Przewalski's horses. In addition, we will compare paired egg and larval samples collected from 10 horse farms in Germany to investigate if and to what extend larval culture has an effect on the results of species composition and quantity detected in a fecal sample using Next Generation Sequencing.

Comprehensive characterization of the P-glycoprotein family in *Parascaris sp.*

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C05 (Associated)

Parasitic nematodes are major pathogens of horses. Chemotherapeutic metaphylaxis remains the most common strategy for helminth control but their frequent and often inappropriate use has driven the development and spread of anthelmintic resistance (AR).
In equines, the development of macrocyclic lactone (ML) resistance in *Parascaris sp*. poses a threat to animal health and welfare. The underlying resistance mechanisms are not well understood but through Next-Generation-Sequencing and target-gene approaches, several genes have emerged as the candidates associated with AR resistance, among them P-glycoproteins (Pgp).
To gain a better understanding of the role of Pgp in ML resistance of *Parascaris sp*. we have characterized the whole family of Pgp genetically and transcriptomically. Using this data as a basis we use several model system to characterize the candidate Pgps at a functional level.
In the cell line LLC-PK1 we aim to establish an assay to directly characterize Pgp transport of different members of the ML group.
To break Pgp-mediated resistance, we try to find inhibitors and modulators of Pgp, that exhibit strong inhibition of nematode Pgp but low inhibition of mammal Pgp in an inhibitory yeast growth assay.
Using the model nematode *Caenorhabditis elegans* and CRISPR/Cas9 genome editing we aim to characterize the role of three single nucleotide polymorphisms in Pgp-11.1 which were previously found to be strongly selected in a resistant population.
So far, we have molecularly characterized 10 Pgp coding sequences in *Parascaris sp*. and we believe that the data from our functional assays will further help to decipher the complex interactions of Pgp and ML.

Manipulation of the Th2/1 to Th2 ratio affects the fitness of parasitic nematodes

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B05 (Gen 1)

Helminths are highly prevalent in livestock and infect a quarter of the human population. The dominant immune response to helminths is characterized by type 2 T helper (Th2) cells, mediating partial immunity to these parasites in the majority of cases. However, reinfections are frequent and the development of protective immunity may take years. Furthermore, there are no vaccines available for the use in humans. Hence, a better understanding of the immune response to helminth infections is needed.

Heligmosomoides polygyrus is a natural mouse parasite serving as a model for gastrointestinal nematodes chronically infecting humans. It induces a strong Th2 response characterized by CD4+ T cells expressing the transcription factor GATA-3 and cytokines including IL-4 and IL-13. We have previously shown that in nematode infections, a substantial fraction of T helper cells differentiates into Th2/1 hybrid cells co-expressing the Th1 lineage specifying transcription factor T-bet and key cytokine IFN-γ along with the Th2 markers. These hybrids developed from naive precursors and similar Th2/1 hybrid to Th2 cell ratios developed in response to immunization with parasite antigens and in germ-free mice, hence their instruction was independent of microbial signals. Mice lacking the IFN-γ receptor displayed a more pronounced conventional Th2 response associated with decreased worm fitness. By contrast, IFN-γ supplementation early during infection supported the differentiation of Th2/1 cells and local as well as systemic IFN-γ production during acute and chronic *H. polygyrus* infection. Importantly, the Th2/1 bias was associated with increased parasite egg production and the maintenance of higher chronic worm burdens. Finally, mouse lines differing in their genetically imprinted susceptibility to worm infections differed significantly in Th2/1 proportions upon *H. polygyrus* infection, but also displayed significant differences in the magnitude of parasite-specific $CD4^+$ T cell responses and the phenotypes of dendritic cells and regulatory T cells. In conclusion, the differentiation of Th2/1 hybrid cells contributes to susceptibility for helminth infections, while the basis for their differential instruction in genetically diverse host populations awaits further investigation.

Publication:

1. **Affinass N**, Zhang H, Löhning M, Hartmann S, Rausch S. (2018). Manipulation of the balance between Th2 and Th2/1 hybrid cell affects parasite nematode fitness. European Journal of Immunology. doi: 10.1002/eji.201847639
2. Rausch S, Midha A, Kuhring M, **Affinass N**, Radonic A, Kühl AA, Bleich A, Renard BY, Hartmann S. (2018). Parasitic nematodes exert antimicrobial activity and benefit from microbiota-driven support for host immune regulation. *Front Immunol*. doi: 10.3389/fimmu.2018.02282

Mechanisms of hybrid T helper cell instruction in nematode infections

Bhavya Kapse
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B05 (Gen 2)

Infections by intestinal helminth parasites are highly prevalent in humans in low income countries and constitute a large economic burden in world-wide livestock production. Immunity against intestinal nematodes centrally involves type 2 helper (Th2) immune responses which eventuate in parasite expulsion and protection against re-infections. However, our group has shown that infections with the natural murine parasite *Heligmosomoides polygyrus* and other helminths are also characterized by the differentiation of Th2/1 hybrid cells which simultaneously co-express the transcription factors and cytokines of Th2 (GATA-3; IL-4/-13) and Th1 (T-bet; IFN-γ) cells, lineages previously considered to be dichotomous. Our group has recently shown that these hybrid cells tip the immune scale in favor of the parasite by increasing parasite fitness and fecundity. Strikingly, the hybrid cells were found to maximally populate the spleen during the course of infection, prompting the question if the spleen favors the instruction of Th2/1 cells and thereby the susceptibility for chronic worm infections.

Recent studies have implicated inflammasome activation during nematode infection in the limitation of protective Th2 responses. The inflammasome catalyzes the activation of caspase 1 further promoting the conversion of pro-IL-1β and pro-IL-18 to their mature bioactive forms. In nematode infection, these cytokines are reported to support IFN-γ production and suppress Th2 immunity.

The aim of the current study is to investigate the role of inflammasome activation in Th2/1 responses, the identification of early IFN-γ sources supporting Th2/1 differentiation and the investigation of the splenic environment as a favorable milieu for differentiation or homing of nematode-associated Th2/1 hybrid cells. Asking if parasitic nematodes directly trigger inflammasome activation, we stimulated murine dendritic cells and macrophages and primary pig lung macrophages with nematode excretory/secretory products. The cellular responses and the in vivo kinetics of inflammasome-associated cytokine production upon infection are currently under investigation. Furthermore, we started to assess the effects of inflammasome blockade on Th2/1 hybrid generation by treating *H. polygyrus* infected mice with inflammasome blocker MCC950 and analyzing the inflammasome-associated cytokine responses and T cell phenotypes.

Phospholipid synthesis in the obligate intracellular parasite Toxoplasma gondii

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A02 (Gen 2)

Toxoplasma gondii is an obligate intracellular parasite, which inflicts acute as well as chronic infections in a wide range of warm-blooded vertebrates. Our recent work has demonstrated the natural occurrence and autonomous synthesis of an exclusive lipid phosphatidylthreonine in *T. gondii*. Targeted gene disruption of phosphatidylthreonine synthase impairs the parasite virulence due to unforeseen attenuation of the consecutive events of motility, egress and invasion. Loss of phosphatidylthreonine depletes calcium stores in intracellular tachyzoites, which leads to dysregulation of calcium release into the cytosol during the egress phase. In this project, we will examine the mechanistic regulation of calcium homeostasis by PtdThr and PtdSer. Upon successful completion, we shall reveal lipid-mediated regulation and mediators of calcium dynamics during asexual reproduction of *T. gondii*, which can eventually be exploited to inhibit the parasite growth.

Development of a non-invasive technique using exfoliated cells to detect infection in mice

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B04 (Gen 1)

Helminth infections occur in both humans and animals. In wildlife, animals are exposed to different pathogens and elicit different immune responses to deal with various infections. Thus, understanding how wild animals interact with a range of pathogens is important especially when access to samples can be difficult. Here, we have developed a non-invasive technique to investigate gene expression using stool samples collected from *H. polygyrus* infected mice. The surface of stool is covered with exfoliated colonic epithelial cells that are shed daily. Here, the exfoliated epithelial cells were removed and RNA was extracted for gene expression analysis using qRT-PCR. During an *H. polygyrus* infection a T helper 2 (Th2) immune response is elicited and various cytokines are released. Additionally goblet cells (GCs) play a role in the secretion of the Th2 effector molecule RELM-β in intestinal tissue that is essential for normal spontaneous worm expulsion. In this study RELM-β mRNA was detectable during acute worm infection and expression levels varied less in exfoliated cells collected in the morning, afternoon and evening compared to egg counts that varied depending on the time of collection. Interestingly we observed a dose-dependent response of RELM-β expression in a low and high dose *H. polygyrus* infection. RELM-β expression was also detectable during a secondary *H. polygyrus* infection after drug treatment. This method was also applicable to wild Mus musculus mice. Wild mice containing worms displayed a significantly higher RELM-β expression compared to mice that did not contain any worms. This study has demonstrated a novel non-invasive technique using exfoliated cells to detect infection in both laboratory mice and wild mice.

Publication:

Ahmed N, French T, Rausch S, Kühl AA, Hemminger K, Dunay IR, Steinfelder S, Hartmann S. (2017). Toxoplasma co-infection prevents Th2 differentiation and leads to a helminth-specific Th1 response. *Front Cell Infect Microbiol* 7:341. doi:10.3389/fcimb.2017.00341.

Macrophage and dendritic cell adaptation to sequential co-infection by protozoan and helminth parasites

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B04 (Gen 2)

In contrast to the Th1 response elicited by *Toxoplasma gondii,* infections by helminths such as *Heligmosomoides polygyrus* and *Schistosoma mansoni* induce a Th2 response, characterized by secretion of IL-4, IL-5, IL-9 and IL-13. However, recent results in our group have demonstrated that in the presence of ongoing *T. gondii* infection, subsequent *H. polygyrus* infection fails to initiate a Th2 immune response. Instead, *H. polygyrus*-specific CD4+ T cells are generated, but these cells express T-bet and IFN-γ; characteristics of Th1 immunity. At present the mechanism which drives this phenomenon is unknown. Furthermore, it is unclear for how long Th2 development against a secondary nematode infection is inhibited.

DCs are central for Th2 differentiation in most helminth infections, and it is known that *T. gondii* heavily manipulates DC function. It is therefore possible that the observed defect in Th2 polarization during *T. gondii-H. polygyrus* co-infection is the result of an altered DC phenotype and modified DC function. One signalling mechanism which could potentially be involved is the PI3K-mTOR pathway. Experiments conducted on mice deficient for PI3K subunit p85α found they exhibited an enhanced Th1 response to infection with the protozoan *Leishmania major*, and a reduced Th2 response to the helminth *Strongyloides venezuelensis*. This seemingly mirrors the Th2 polarization defect observed in our co-infection model and warrants further investigation.

Another aspect to consider is that in recent years significant differences have been identified between the immune responses of mice and humans to *T. gondii.* Likewise, helminths are highly host specific, with most worm species infecting only one or a small number of closely related host species. At present the majority of research conducted into both *T. gondii* and helminth immunology utilizes murine models. However, given the significant host-specific differences, it may be beneficial to establish a more human-like model.

Antimicrobial activities of excreted/secreted products of intestinal nematodes

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B04 (Associated)

Intestinal nematodes infect over a billion people and also pose an enormous economic burden for livestock producers. In addition to modulating the immune systems of their hosts, helminths reside amongst the gut microbiota, an environment that likely poses immense microbial-infectious challenges for the nematodes. Furthermore, nematode infections have been shown to alter the composition of the host-intestinal microbiota. This close association between nematodes and microbes suggests that helminths have evolved antimicrobial strategies ensuring their long-term survival within their hosts, though little is known about these interactions. Thus, we hypothesize that parasitic nematodes release factors with antimicrobial activities. To test this hypothesis, excreted and secreted (ES) products were collected from *ex vivo* cultured nematodes *(Ascaris suum and Heligmosomoides polygyrus)* and characterized. ES products were sequenced by liquid chromatography-mass spectrometry as well as employed in a variety of antimicrobial and anti- biofilm assays. Preliminary results demonstrate potent antimicrobial activity against gram-positive and gram-negative organisms, as well as activity against biofilm-forming *E. coli* strains. Peptide sequencing has also identified several proteins and peptides with known or predicted antimicrobial activity. ES products from both nematode species possess bacterial-agglutinating activity, likely due to the presence of C-type lectin domain-containing proteins. Ongoing studies are aimed at characterizing one such C-type lectin protein detected in *A. suum* ES products as well as assessing the impact of an *A. suum* infection on the composition of the porcine microbiome.

Publications:

1. Rausch S, **Midha A**, Kuhring M, Affinass N, Radoncic A, Kühl AA, Bleich A, Renard BY, Hartmann S. 2018. Parasitic nematodes exert antimicrobial activity and benefit from microbiota-driven support for host immune regulation. Front Immunol, doi: 10.3389/fimmu.2018.02282.
2. Ebner F, Kuhring M, Radoncic A, **Midha A**, Renard BY, Hartmann S. 2018. Silent witness: dual-species transcriptomics reveals epithelial immunological quiescence to helminth larval encounter and fostered larval development. Front Immunol, doi: 10.3389/fimmu.2018.01868.
3. **Midha A**, Janek K, Niewienda A, Henklein P, Guenther S, Serra DO, Schlosser J, Hengge R, Hartmann S. The intestinal round work Ascaris suum releases antimicrobial factors which interfere with bacterial growth and biofilm formation. Front Cell Infect Microbiol, doi: 10.3389/fcimb.2018.00271.
4. **Midha A**, Schlosser J, Hartmann S. 2017. Recipricol interactions between nematodes and their microbial environments. Front Cell Infect Microbiol, doi: 10.3389/fcimb.2017.00144.

Identifying cat transmission-relevant rodent reservoirs for virulent *Toxoplasma gondii* strains by analyzing IRGs-mediated resistance mechanisms

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C03 (Gen 1)

One *Mus musculus* resistance mechanism to infection with virulent type I *Toxoplasma gondii* strains relies on the polymorphic Immunity-Related GTPase *Irgb2-b1*, induced by IFN-γ. However, studies in Europe show that cats prey more on other rodent species, such as the voles *Myodes glareolus*, *Microtus* spp. and the field mice *Apodemus* spp., which also show higher *T. gondii* seroprevalences compared to *Mus* spp. We aim at assessing whether specific *Irg* sequences confer resistance to type I *T. gondii* infection in these wild rodent species. The results will help defining ecologically important intermediate hosts for virulent parasite transmission to cats. Having access to a large collection of tissue samples across Germany from *M. glareolus, Microtus* spp. and *Apodemus* spp., we first confirmed wild rodents' genetic variability of IRGb2-like and IRGb1-like proteins, especially in residues in the IRGb2-like subunit at the putative interface with T. gondii virulence effector ROP5, similarly to wild-derived *Mus* spp. We then showed that *M. glareolus* bone marrow-derived macrophages (BMDM), primary fibroblasts and kidney cells undergo IFN-γ-dependent necrosis following type I infection. This phenotype was proposed to be associated with host resistance by limiting parasite replication. Furthermore IFN-γ-treated BMDM from *M. glareolus* reduce virulent parasite burden compared to untreated cells, suggesting an IFN-γ-mediated host resistance mechanism. In order to assess whether the IRGb2-b1-like protein is responsible for this phenotype, similarly to what observed in wild-derived *Mus* spp., we have set up an expression system that will allow us to express wild rodents' Irg-like genes in lab mice-derived Flp-In-3T3 fibroblasts susceptible to infection with virulent *T. gondii* strains. Following infection with type I *T. gondii* we will observe whether the introduced sequences lead to parasite death via disruption of the parasitophorous vacuole membrane (PVM).As reference we cloned *Irgb2-b1*-like cDNAs of respective rodent cell lines available to us, i.e. BVK168 *M. glareolus,* FMN-R Microtus arvalis and AAL-R *Apodemus agrarius*, after induction with either our custom-produced recombinant vole IFN-γ (MgIFN-γ) or mouse IFN-γ, respectively. We showed that wild rodents'-derived IRGb2 subunit from reference cell lines is sufficient to bind virulent parasites' PVM in established stable Flp-In 3T3 cell lines. Initial results indicate the suitability of our created cell lines for studying the role of wild rodent IRGb2-b1-like proteins on infection by virulent *T. gondii.* Phenotypic characterization of the established clones is currently ongoing. Results will help to assess the ecological importance of wild rodents as intermediate hosts for virulent parasite transmission to cats.

Publications:

1. Ehret, T., **Torelli, F.,** Klotz, C., Pedersen, A. B., Seeber, F. Translational Rodent Models for Research on Parasitic Protozoa - A Review of Confounders and Possibilities. Front. Cell. Infect. Microbiol. 7, 238 (2017).
2. **Torelli,** F., Zander, S., Ellerbrok, H., Kochs, G., Ulrich, R.G., Klotz, C., Seeber, F. Recombinant IFN-γ from the bank vole Myodes glareolus: a novel tool for research on rodent reservoirs of zoonotic pathogens. Sci. Rep. 8, 2797 (2018).
3. Ferreira S.C.M. *, **Torelli F.** *, Klein S., Fyumagwa R., Hofer H., Seeber F., East M.L. Seroprevalence of Toxoplasma gondii in African carnivores in East Africa. Int J Parasitol Parasites Wildl (**in revision**) (2018) (* shared first authorship)

Innate immune responses of intestinal organoids from wild rodents upon *Toxoplasma gondii* infection

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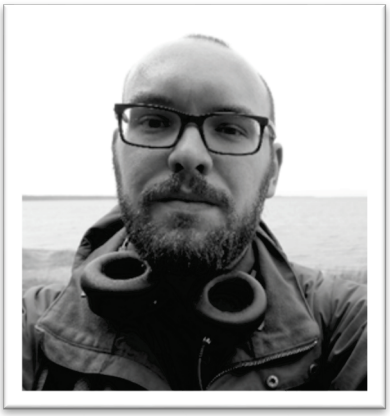
C03 (Gen 2)

Toxoplasma gondii's route of infection is via the oral/intestinal route, however current knowledge of how this proceeds and which innate immune factors are involved is only vaguely known. Furthermore, almost all studies on intestinal T. gondii infections were done in C57/BL6 mice since they are highly susceptible to oral infection by cysts, which results in gut pathology. However, BALB/c mice are resistant under the same conditions, indicating large differences even in the same host species. Additionally, wild house mice and wild rodents are largely understudied and little is known about *T.gondii* early infection events in these animals considering their ecological relevance in parasite transmission.

Our aim is to study the infection with *T. gondii* of intestinal organoids from wild rodents in comparison to lab mouse organoids with regard to intestinal innate immune responses. This comparison will be carried out by live-cell microscopy, cytokine assays, and transcriptomics. We have successfully generated intestinal organoids from the wild rodent species *Myodes glareolus* and had them in culture for more than four months. During this time we optimized growth conditions for future experiments, with the goal of generating differentiated organoids that contain the multiple cell lineages of intestinal epithelium such as paneth and goblet cells.

We have carried out several pilot *toxoplasma* infection experiments in our organoid model, to evaluate the suitability of the model to study infection. The next step is to conduct these infections using live-cell microscopy, in order to characterize the events during the initial stages of intestinal infection. We plan to use the organoid model to evaluate the effects of different *T. gondii* stages (tachyzoites, bradyzoites; possibly oocysts) on the development of innate immune responses, by comparing responses elicited in inbred laboratory mice and wild rodents under stimulation of IFN-γ.

Oocyst-molecules of *Toxoplasma gondii:* Their role for survival in the environment and their diagnostic potential



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C03 (Associated)

It is assumed that oocysts from *T. gondii* play a key role in spreading of the parasite. To improve detection of oocysts in environmental samples, we aim to develop camelid single-domain antibodies (nanobodies) to efficiently extract oocysts from such samples and analyse them by subsequent PCR. To achieve this a cDNA-derived phage display library from an Alpaca immunised with oocysts from *T. gondii* is established. Recent immunofluorescence assays seem to confirm a successful immunization.

In a second project, we aim to characterize proteins expressed in the oocysts to determine their role in the survival of oocysts in the environment. These proteins seem to belong to the class of Late Embryogenesis Abundant (LEA) proteins which are known for their potential to protect organisms against harsh environmental conditions such as desiccation or low temperatures. To assess the protecting potential of the LEA proteins, growth assays in *Escherichia coli* and *Saccharomyces cerevisae* under different stressing conditions have been conducted and analyzed. First results show that at least one of the investigated LEA-proteins contributes to desiccation tolerance in *E. coli.*

In a third project we examine the potential of these LEA-proteins to serve as a marker for an oocyst conveyed infection.

Shifts in Treg/Th17 balance correlate with differential control of infection with *Giardia muris*

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B05 (Associated)

Infection with *Giardia lamblia* remains among the most common causes of food- and water-borne diarrhoeal disease in the world, with an estimated global prevalence of 184 million cases. *Giardia* are binucleated flagellates with a direct life cycle and only two developmental stages – infective cysts and the disease-causing trophozoite stage, colonizing the small intestinal tract of the host. Host protection against the parasite relies on intestinal IgA secretion and adaptive Th17 responses. Early studies have demonstrated a difference in susceptibility to infection with the mouse species *Giardia muris* between inbred mouse lines, such as BALB/c and C57BL/6 mice. Recently these mouse strains have also been demonstrated to differ in the induction and maintenance of adaptive intestinal Th17 responses, with C57BL/6 mice displaying more potent Th17 responses under homeostasis. The development of RORγT$^+$ Th17 cells expressing IL-17A is closely linked to that of Foxp3$^+$ regulatory T cells. We set out to investigate the dynamics of Treg/Th17 responses in BALB/c and C57BL/6 mice infected with *Giardia muris* to establish whether the development of a Treg/Th17 imbalance exerts an influence on the control of giardiasis. Expectedly, BALB/c mice infected with *G. muris* shed more cysts compared to C57BL/6 mice. This correlated with more prominent intestinal IL-17A responses in infected C57BL/6, but not BALB/c mice. By contrast, infected BALB/c mice displayed elevated frequencies of Foxp3$^+$ regulatory T cells in the small intestine compared to infected C57BL/6 mice. More importantly, many more BALB/c Foxp3$^+$ cells expressed RORγT, a phenotype previously reported for highly active regulatory T cells. Furthermore, our data suggest a positive correlation between Treg/Th17 ratios and cyst excretion rates in *G. muris*-infected mice. As the microbiota composition of BALB/c and C57BL/6 mice differed in steady state and upon *G. muris* infection, we performed co-housing experiments. Our preliminary data suggest that neither the differential intestinal Th17 nor Treg responses depend on microbiota differences between the mouse lines. It hence seems that susceptibility to *Giardia* infection is associated with a microbiota-independent bias and that the induction of Treg responses could be contributing to higher susceptibility to infection with *Giardia* in mice.

Publication:

Yordanova IA*, Zakovic S*, Rausch S, Costa G, Levashina E, Hartmann S (2018). Micromanaging immunity in hosts and vectors: microbiota-dependent immune responses to intestinal parasites. *Front Cell Infect Microbiol*.doi:*10.3389/fcimb.2018.00308*

Influences of metabolic stress on *Giardia duodenalis* growth

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A04 (Gen 1)

Giardia duodenalis infects the intestinal epithelium of ~280 million humans. This unicellular parasite adheres to epithelial cells in its trophozoite stage and at a given time converts into a cyst. Symptoms vary from asymptomatic to chronic infections with pain, diarrhea, and risk of malnutrition. Several reports suggest that arginine may be a modulator of *G. duodenalis* virulence. Firstly, the parasite possesses an enzyme shared with many bacteria, arginine deiminase (ADI), which is central in a pathway for utilizing arginine as energy source. In *G. duodenalis,* the catabolism of arginine potentially fuels ATP synthesis more efficiently than glucose. Secondly, in vitro ADI is upregulated and released when parasites come into contact with host cells. Third, arginine is an interesting metabolite to investigate, due to its potential to influence host immune responses. Arginine is a substrate for nitric oxide (NO) which is an important innate defense against microorganisms. In addition, amino acids activate mTOR signalling which, e.g., has an impact on T-cell regulation. I speculate that poor or no access to arginine will influence parasite fate-decisions to either replicate as a trophozoite or to form cysts. Our estimates suggest that *G. duodenalis* through the high number of parasites may deplete luminal arginine in the host, potentially providing a danger signal to the host.

The in vivo relevance of arginine during *G. duodenalis* infection has so far not been investigated. I established a mouse - *G. duodenalis* infection model, utilizing intestinal epithelium-specific mTOR (loxP/Cre) knock-out mice, which are bred in-house. These mice will allow targeted investigation of mTOR-dependent host epithelial responses during infection, which will be analyzed by dual (host and parasite) RNA sequencing. Furthermore, I can detect and quantify cysts in feces with comparatively high throughput, which is important considering the relatively high number of replicates (15 mice per group). Cyst quantification will be compared to *Giardia*-qPCR data from feces as an indication of total parasite numbers. The ratio provides a proxy for cyst-conversion rates in different experimental groups. Using the infection model described above, I will manipulate the access to arginine in the host. Taken together, the described experiments provide data to address 1) whether access to arginine in the intestinal lumen influences *G. duodenalis* trophozoite fate-decisions to either replicate or form cysts, and 2) host transcriptional responses to differences in luminal arginine levels caused either by *G. duodenalis* or by no/low intake via the food.

Publications:

1. **Ehret, Totta**, et al. "Dual RNA-seq reveals no plastic transcriptional response of the coccidian parasite Eimeria falciformis to host immune defenses." *BMC genomics* 18.1 (2017): 686.
2. **Ehret, Totta**, et al. "Translational rodent models for research on parasitic protozoa—a review of confounders and possibilities." *Frontiers in cellular and infection microbiology* 7 (2017): 238.

Human small intestinal organoids – a superior model to investigate *Giardia* sp. Infection

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B03 (Gen 1)

Giardia duodenalis is one of the most abundant parasites, responsible for over 280 million cases of the enteric disease “giardiasis” every year, worldwide. One proposed pathomechanism is the induction of epithelial barrier dysfunction by apoptosis or tight junctional alterations, which increase epithelial permeability, may impact nutrient uptake and normal gut function, or even lead to invasion by luminal bacteria. However, our studies on the well-known and established Caco-2 *in vitro* co-culture system did not show any barrier destructing effects of eleven different Giardia sp. isolates under various infection conditions. As a new approach, human small intestinal organoids, stem cell-derived *in vitro* miniature versions of the gut, were used as a more sophisticated model system. By generating small intestinal epithelial monolayers from stem cell enriched organoid cultures we were able to set up a model which reproduces the *in vivo* epithelium unlike any cancer-based cell line system. In contrast to our *Giardia* sp. – Caco-2 infection experiments, the infection of organoid-derived monolayers allowed conditions in which the parasite is reproducibly able to destroy the integrity of monolayers, leading to barrier dysfunction. With this new model, the parasite’s pathomechanism is currently investigated in more detail.

Publication:

Kraft MR, Klotz C, Bücker R, Schulzke J-D and Aebischer T (2017) *Giardia's* Epithelial Cell Interaction *In Vitro*: Mimicking Asymptomatic Infection? *Front. Cell. Infect. Microbiol*. 7:421. doi: 10.3389/fcimb.2017.00421

Characterisation of antibody responses against GPI-epitopes

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A03 (Gen 1)

P. falciparum-specific glycosylphosphatidylinositol (PfGPI) molecules can activate pathways of the inflammatory cascade which contribute to severe malaria pathogenesis. Anti-PfGPI antibodies have been detected in people living in malaria-endemic areas. It is postulated that these antibodies, or a subset thereof, play a role in protecting individuals from severe malaria. However, their effector function and epitope specificity are not fully understood.
A PfGPI minimal epitope antigen was designed, synthesized and formulated into a glycoconjugate. Immunisation of mice with this glycoconjugate successfully induced epitope-specific antibodies. A follow-up challenge study using the experimental cerebral malaria mouse model showed that glycoconjugate immunisation did not protect against severe malaria pathogenesis. A monoclonal antibody cell line was produced from immunized mice to further characterize the effector function of the epitope-specific antibodies.
The implications for anti-toxin vaccine synthesis and formulation using PfGPI glycoconjugates will be evaluated.

Publication:

Jaurigue JA, Seeberger PH. (2017). Parasite Carbohydrate Vaccines. *Front Cell Infect Microbiol* 7:248. doi:10.3389/fcimb.2017.00248. Review article

Characterization of *Plasmodium falciparum* artemisinin resistance in southern Rwanda, 2010-2018

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B02 (Gen 1)

Artemisinin-based combination therapy (ACT) is the mainstay of malaria treatment and control. However, emerging resistance of *Plasmodium falciparum* to artemisinin derivatives (ART) in the Greater Mekong Sub-region and recently in Uganda may threaten the achievements of the last decade in reducing malaria morbidity and mortality.

Surveillance for ART resistance has been facilitated by the discovery of the molecular marker Kelch 13 *(K13)* and the development of a simple *ex vivo* ring stage survival assay (RSA) that by mimicking clinical drug exposure *in vitro*, can provide a correlation between *K13 mutants* and delayed parasite clearance observed *in vivo* and thereby it adds to traditional *in vitro* susceptibility testing.

In our prior work in the Huye district in southern Rwanda, five K13 single nucleotide polymorphisms were identified in *P. falciparum* isolates collected between 2010 and 2015, two of which are associated with ART resistance in Southeast Asia. This suggests the presence of ART tolerance or resistance in the Huye district.

The aim of the current project is to confirm or refute the presence of ART resistance in the study area by improving the current knowledge on the prevalence and characteristics of ART-resistant *P. falciparum* isolates in the Huye district of southern Rwanda by assessing K13 variants, by conducting RSA and *in vitro* assays, and by relating molecular and *ex/in vitro* findings.

Publications:

1. **Tacoli C,** Gai PP, Bayingana C, Sifft K, Geus D, Ndoli J, Sendegeya A, Gahutu JB, Mockenhaupt FP. Artemisinin resistance-associated K13 polymorphisms of Plasmodium falciparum in southern Rwanda, 2010-2015. Am J Trop Med Hyg. 2016 Nov 2;95(5):1090-1093. Epub 2016 Aug 29.
2. Esu E, **Tacoli C,** Gai PP, Berens-Riha N, Pritsch M, Loescher T, Meremikwu M. Prevalence of the Pfdhfr and Pfdhps mutations among asymptomatic pregnant women in Southeast Nigeria. Parasitol Res. 2018 Mar;117(3):801-807. doi: 10.1007/s00436-018-5754-5. Epub 2018 Jan 13.
3. <in press> Jakob W, **Tacoli C**, Gai PP, Siegert K, Kulkarni S, Rasalkar R, Boloor A, Jain A, Mahabala C, Baliga S, Shenoy D, Devi R, Gai P, Mockenhaupt FP. Molecular evidence for Plasmodium falciparum resistance to sulfadoxine-pyrimethamine but absence of K13 mutations in Mangaluru, southwestern India. Am J Trop Med Hyg. 2018 Oct;00(0):1-3.

Association between α-thalassemia and artemisinin effectivity on *Plasmodium falciparum in vitro* and in the field

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B02 (Gen 2)

In vitro experiments in the ’80s and ’90s have shown that the antimalarial drug artemisinin is less effective in the case of erythrocyte variant α-thalassemia. Artemisinin is the first line drug for *P. falciparum*; a parasite causing an estimated 90% of malaria infections per year on the African continent. A very mild form of α-thalassemia is estimated to be present in 30-60% of the Sub-Sahara African population. Reduced drug pressure in α-thalassemic malaria patients might affect the direct treatment efficacy and the development of resistant parasite strains. We aim to investigate the impact and relevance of α-thalassemia for artemisinin efficacy on *P. falciparum* clearance by addressing three main questions:

1. Do *P. falciparum* field strains have a different IC50 in an *in vitro* susceptibility assay with artemisinin when comparing α-thalassemic erythrocyte variants with normal erythrocytes?
2. Does artemisinin-based treatment outcome differ for malaria patients with haemoglobinopathies and for malaria patients without erythrocyte variants?
3. Does *P. falciparum* develop artemisinin resistance in a different pace in α-thalassemic erythrocyte variants than in normal erythrocytes in a long-term *in vitro* culture?

Pre-clinical evaluation of a triple genetically arrested parasite (tKO GAP) for malaria vaccine development

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B01 (Gen 1)

Attacking and eliminating *Plasmodium* parasites in the liver prior to the symptomatic blood infection is one of the most promising malaria vaccine strategies. A number of genetically arrested parasites (GAPs) have been engineered in *Plasmodium berghei, P. yoelii* and *P. falciparum* with varying safety and efficacy. One GAP-vaccine candidate, sporozoites containing a targeted deletion of the master regulator of liver stage development *SLARP*, provides the most robust life cycle arrest *in vivo* and *vitro* and, hence, fulfill all safety requirements. However, immunizations with Δ*SLARP* sporozoites do not elicit long-lasting immunity, most likely as a result of early liver stage arrest. On the other end of the spectrum, Δ*P36p* sporozoites elicit long-lasting immunity, but lead to break-through infections during immunizations.

Here, we combined the two knockouts Δ*SLARP* and Δ*P36p/P36* and present a systematic pre-clinical evaluation of this tKO GAP parasite line, which is a crucial step before translation to human clinical trials. We show complete arrest of Δ*SLARP/P36p/P36* parasites in cultured hepatoma cells and sporozoite-infected mice. We analyzed these tKO GAP sporozoites for their immunogenicity and their potential to induce protection in comparison to the respective single knockout lines as well as irradiated sporozoites (γspz). Animals immunized with Δ*SLARP/P36p/P36* parasites elicit similar antibody titres and numbers of IFNγ-producing CD8+ T-cells as mice immunized with single knockout sporozoites. Parasite load in the liver and the time to blood infection after a challenge sporozoite infection were measured to estimate the potency of the tKO GAP vaccine candidate. tKO GAPs serve as a platform towards safer and more immunogenic whole sporozoite vaccine candidates.

Publication:

Kreutzfeld O, Müller K, Matuschewski K. (2017). Engineering of genetically arrested parasites (GAPs) for a precision Malaria vaccine. *Front Cell Infect Microbiol* 7:198. doi: 10.3389/ fcimb.2017.00198

Expression of *Salmonella enterica* flagellin in transgenic *Plasmodium berghei* as adjuvant to potentially improve malarial vaccine efficacy

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B01 (Gen 2)

The development of an efficacious vaccine capable of providing adequate protective immunity against *Plasmodium* parasites still remains as a major challenge despite countless effort over the years.

Here, we generated new transgenic lines of *Plasmodium berghei* that express the flagellin of *Salmonella enterica* Serovar Typhimurium, either with or without an export peptide, as a carry - along adjuvant to boost the immune responses triggered by live-attenuated *Plasmodium* parasites. This bacterial flagellin has an intrinsic innate immunity-stimulating activity mediated through toll-like receptor 5 (TLR-5), and is therefore a promising candidate for an effective vaccine adjuvant conferring enhanced antibody and T cell responses. According to positive results from previous studies with recombinantly expressed *Plasmodium* antigens coupled to flagellin, it can be hypothesized that there will be an improvement in protective immunity in murine models immunized with whole parasite vaccines used in conjunction with this adjuvant. So far, we have demonstrated that the expression of flagellin is possible during the sporozoite, liver, and blood stages of the parasite. However, sporozoite prime-boost immunizations in murine models using *P. berghei* expressing flagellin with export peptide did not show improved protection as compared to wild-type *P. berghei*. Future work will therefore investigate the approach of using *P. berghei* expressing flagellin without export peptide for immunization to promote protective immunity against blood infection.

The role of *Plasmodium falciparum* derived microvesicles in malaria related anemia

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B01 (Associated)

Severe anemia represents one of the major complications in malaria infection affecting mostly young children. While it is evident that the invasion of a red blood cell (RBC) by the malaria parasite directly implies its subsequent destruction, it is well established that when compared to the actual level of parasitemia a disproportional amount of RBCs is lost during infection. To date there is no conclusive mechanism explaining this additional loss of non-infected RBCs. However during *Plasmodium* infection, parasitic material is released massively into the blood stream in multiple forms, including parasite-derived microvesicles, and might end up on host cells, including non-infected RBCs. In addition, in response to *Plasmodium* the host immune system produces a multitude of antibodies directed against parasitic antigens. These antibodies might mediate anemia if they bind to their cognate antigens on the surface of non-infected RBCs by initiating phagocytosis or the complement cascade. Our aim is to investigate the contribution of the transferred parasitic material to naïve RBCs in the development of malaria-dependent anemia. We purify and characterize microvesicles from *Plasmodium falciparum* cultures by mass spectrometry, electron microscopy and western blot ; their transfer to the surface of non-infected RBCs is assessed by immunofluorence assay. We show, using the ring infected erythrocyte surface antigen (RESA) as reporter protein, that parasitic antigens are transferred to the surface of uRBCs via microvesicles and that this transfer primes uRBCs for destruction *in vitro* by macrophages. Our findings show that extracellular vesicles contribute to the pathogenesis of malaria related anemia by transferring parasite antigens to uRBCs.

Publication:

Kapishnikov S, Leiserowitz L, Yang Y, Cloetens P, Pereiro E, **Awamu Ndonglack F**, Matuschewski K, Als-Nielsen J. (2017). Biochemistry of malaria parasite infected red blood cells by X-ray microscopy. *Sci Rep* 7:802.

Role of REL2/IMD pathway in the vectorial capacity of malaria mosquito *Anopheles gambiae*

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A01 (Gen 1)

Malaria vectors, *Anopheles* mosquitoes, make one of the deadliest animals in the world. Although vast effort has been placed to identify and exploit mechanisms behind malaria killing in the mosquitoes for transmission control strategies, these mechanisms remain largely unknown.
Nevertheless, NF-κB pathway REL2 was discovered as an important regulator of immune responses to human malaria parasites, as its experimental activation renders mosquitoes resistant to *P. falciparum*. In order to identify the mechanisms behind the REL2 specificity of *Plasmodium* killing, we established loss-of-function mutants of the NF-κB transcription factor REL2 using CRISPR-Cas9 system ($REL2^{-/-}$). Using these mutants, we began investigating the role of REL2 pathway in the vectorial capacity of *A. gambiae*. Surprisingly, we observed an inconsistent and often more resistant phenotype of the REL2-/- mutants upon *P. falciparum* infection, which was non concordant with previous studies. We hypothesize that composition of microbiota is affecting the parasite development and susceptibility of the mosquitoes to *Plasmodium.*
We aim to uncover the role of microbiota and REL2 signaling in *Plasmodium* infections, which may allow us to identify news targets to combat malaria.

Publications:

1. **Zakovic S,** Levashina EA. (2018). Insects Go on a STING Operation to Tackle Intracellular Invaders. *Immunity*.49:195-7. doi: 10.1016/j.immuni.2018.08.003.
2. **Zakovic S**, Levashina EA. (2017). NF-κB-Like Signaling Pathway REL2 in Immune Defenses of the Malaria Vector Anopheles gambiae. *Front Cell Infect Microbiol* 7:258. doi: 10.3389/ fcimb.2017.00258.
3. Yordanova IA*, **Zakovic S***, Rausch S, Costa G, Levashina E, Hartmann S (2018). Micromanaging immunity in hosts and vectors: microbiota-dependent immune responses to intestinal parasites. *Front Cell Infect Microbiol.* doi:10.3389/fcimb.2018.00308

The role of microbial communities in structuring mosquito populations in Mali

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A01 (Gen 2)

Malaria is one of the most life-threatening tropical diseases and causes nearly half a million deaths per year whereby the most prevalent area for Malaria is sub-Saharan Africa. Only certain types of mosquitoes are susceptible to *Plasmodium* parasites. And for those, several factors regulate the parasite development within the mosquito.

There are some studies, which show that the tripartite interplay of microbiome, the immune system of the mosquito and *Plasmodium* likely shape mosquito susceptibility to malaria parasites. In this interaction, the microbiome not only exerts a strong effect on the parasite, but also on the vector itself and is likely to structure mosquito populations.

Because there is only little known about mosquito-bacteria interaction, we examine the role of microbiota in structuring populations of the mosquito larvae by doing high throughput time series analyses of four individual larval stages and pupae stage from two consecutive rainy seasons from Mali. Moreover, the dynamics of microbiota composition throughout the rainy season has never been explored.

The obtained results will be used to uncover relationships between microbiome composition of larval stages, sex, mosquito species, *TEP1* genotypes and their evolution during the rainy season. Ultimately, the analyses should identify drivers of microbiome composition and provide new insights in how they affect the structuring of mosquito populations.

Flourishing in germs: Deciphering the role of bacteria in development of the malaria vector *Anopheles gambiae*

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A01 (Associated)

In Africa, *Anopheles gambiae* s.l. mosquito is the major malaria vector. This vector harbours a wide range of microbes, mainly bacteria. These microbes have been associated with different physiological processes in the mosquito such as development, growth, reproduction and vector competence. Of great significance is the role of the gut microbiota in larval development, lack of which leads to arrested development. Development is restored by association with various bacterial species, indicating that the bacterial factors required for development are conserved and produced by many bacteria species. Using a systematic screen of the whole genome knock out library of *E. coli* mutant strains (Keio collection), we identify bacterial genes that play a role in mosquito development. Our results suggest two possible functions of bacteria in mosquito development: (1) metabolic function, particularly provision of aromatic amino acids and (2) signaling function that could be achieved through metabolites or mediated by bacterial flagella. We will next investigate which pathways are activated in the mosquito by the bacteria derived aromatic amino acids and the flagella. Deciphering the essential requirements for *Anopheles* development is crucial for a better understanding of the environmental demands of *Anopheles* and should provide new insights into how the environmental microbiota shapes population structures of *Anopheles.*

Publication:

Kiuru CW, Oyieke FA, Mukabana WR, Mwangangi J, Kamau L, Muhia-Matoke D. (2018). Status ofinsecticide resistance in malaria vectors in Kwale County, Coastal Kenya. *Malar J* 17:3

Coming soon!

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