WELCOME

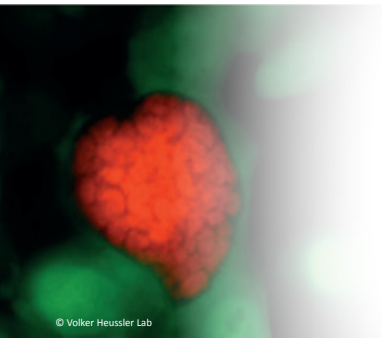
DGP 18 BERLIN

Freie Universität Berlin

28th ANNUAL MEETING OF THE GERMAN SOCIETY FOR PARASITOLOGY

21–24 March 2018

www.parasitology-meeting.de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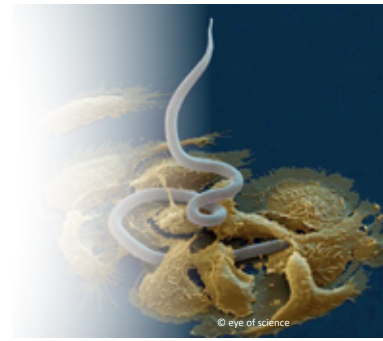
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PROGRAMME

BEIDE HÄLFTEN IM BLICK.

NEU: mit Lungenwurm-prophylaxe

NexGard SPECTRA: WIRKT GEGEN INNERE UND ÄUSSERE PARASITEN.

Die besten Lösungen behandeln beide Seiten des Problems: Kombinierter Endekto-Schutz in einer leckeren Kautablette

NexGard SPECTRA™

- **SchutzPlus:** Wirkung gegen Rundwürmer,* Zecken & Flöhe
- **Herz- und Lungenwurmprophylaxe**
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* Wirkung gegen Endoparasiten (adulte Formen folgender Magen-Darm-Nematoden): Spulwürmer *(Toxocara canis* und *Toxascaris leonina)*, Hakenwürmer *(Ancylostoma caninum*, *Ancylostoma braziliense)* und Peitschenwürmer *(Trichuris vulpis)*; beugt Herzwurmerkrankungen *(Dirofilaria immitis)* vor durch Wirkung gegen Larven; zur Vorbeugung der Angiostrongylose (durch Verringerung des Befalls mit präadulten (L5) und adulten Stadien von *Angiostrongylus vasorum).*

[1] Die empfohlene Dosis sollte bei Collies oder damit verwandten Rassen streng eingehalten werden.

NexGard SPECTRA Kautabletten für Hunde 2–3,5 kg, > 3,5–7,5 kg, > 7,5–15 kg, > 15–30 kg, > 30–60 kg. **Zusammensetzung:** 1 Kautablette enthält: Hunde 2–3,5 kg: 9,375 mg Afoxolaner, 1,875 mg Milbemycinoxim. Hunde > 3,5–7,5 kg: 18,75 mg Afoxolaner, 3,75 mg Milbemycinoxim. Hunde > 7,5–15 kg: 37,5 mg Afoxolaner, 7,50 mg Milbemycinoxim. Hunde > 15–30 kg: 75,0 mg Afoxolaner, 15,00 mg Milbemycinoxim. Hunde > 30–60 kg: 150,00 mg Afoxolaner, 30,00 mg Milbemycinoxim. **Anwendungsgebiete:** Zur Behandlung eines Floh- und Zeckenbefalls bei Hunden, wenn gleichzeitig eine Vorbeugung der Herzwurmkrankheit *(Dirofilaria immitis* Larven), Angiostrongylose (Verringerung des Befalls mit präadulten (L5) und adulten Stadien von *Angiostrongylus vasorum)* und/oder eine Behandlung gegen Magen-Darm-Nematoden angezeigt ist. Zur Behandlung eines Flohbefalls *(Ctenocephalides felis und C. canis)* bei Hunden für 5 Wochen. Zur Behandlung eines Zeckenbefalls *(Dermacentor reticulatus, Ixodes ricinus, Rhipicephalus sanguineus)* bei Hunden für 4 Wochen. Flöhe und Zecken müssen am Wirtstier anheften und mit der Nahrungsaufnahme beginnen, um dem Wirkstoff ausgesetzt zu werden. Zur Behandlung des Befalls mit adulten Formen folgender Magen-Darm-Nematoden: Spulwürmer *(Toxocara canis* und *Toxascaris leonina),* Hakenwürmer *(Ancylostoma caninum, Ancylostoma braziliense* und *Ancylostoma ceylanicum)* und Peitschenwürmer *(Trichuris vulpis).* Zur Vorbeugung der Herzwurmkrankheit *(Dirofilaria immitis* Larven) mit monatlicher Verabreichung. Zur Vorbeugung der Angiostrongylose (durch Verringerung des Befalls mit präadulten (L5) und adulten Stadien von *Angiostrongylus vasorum)* mit monatlicher Verabreichung. **Gegenanzeigen:** Nicht anwenden bei bekannter Überempfindlichkeit gegenüber den Wirkstoffen oder einem der sonstigen Bestandteile. **Nebenwirkungen:** In klinischen Untersuchungen wurden keine schwerwiegenden Nebenwirkungen beobachtet, die auf die Kombination Afoxolaner und Milbemycinoxim zurückzuführen waren. Gelegentlich wurden Nebenwirkungen wie Erbrechen, Durchfall, Apathie, Appetitlosigkeit und Juckreiz beobachtet. Diese Erscheinungen waren in der Regel selbstlimitierend und von kurzer Dauer. **Verschreibungspflichtig. Pharmazeutischer Unternehmer:** Boehringer Ingelheim Vetmedica GmbH, D-55216 Ingelheim. [10.2017]

Boehringer Ingelheim

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ISBN 978-3-9816508-7-7

ORGANISATION AND IMPRINT

Venue (Conference, 21–23 March)
Freie Universität Berlin
Henry Ford Building

Garystraße 35
14195 Berlin l Germany

Venue (Workshops, 24 March)
Freie Universität Berlin
Institute of Parasitology and Tropical Veterinary Medicine
Robert-von-Ostertag-Straße 7–13
14163 Berlin I Germany

Conference website
www.parasitology-meeting.de

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Freie Universität Berlin
Director
Institute of Parasitology and Tropical Veterinary Medicine
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Design/layout

Layout	Krea.tif-art UG (haftungsbeschränkt)
Print	siblog – Gesellschaft für Dialogmarketing, Fulfillment & Lettershop mbH
Circulation	500
Editorial Deadline	2 March 2018

Deutsche Gesellschaft für Parasitologie e. V.
Schriftführer/Schatzmeister
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WELCOME NOTE

Dear Colleagues,
Dear Guests,

On behalf of the German Society for Parasitology (DGP) and the scientific committee cordially invite you to the 28th DGP meeting.

Research in parasitology continues to be a vivid field of life sciences with many exciting new findings and important challenges. This includes not only basic investigations on the biological processes occurring during the parasite-host encounter, the establishment and reproduction of parasites in various environments, but also applied studies on the treatment and control of parasitic infections. Traditionally, the biannual scientific conference of the DGP offers a platform for a broad spectrum of contemporary parasitological topics and research questions. This will also be the case at the next meeting, which will be from the 21st through to the 24th of March in Berlin. The scientific session will be held at the 'Henry-Ford Bau' on the main campus of the Freie Universität Berlin in Dahlem. On the 24th of March two specific workshops will be offered on 'artificial tick feeding' and on a 'disease vector map and distribution model software (VECMAP)' at the campus of the veterinary department of Freie Universität Berlin. For conference participants the attendance at these workshops is free of charge.

In addition to various oral and poster presentation sessions, there will be a range of invited lectures held by eminent scientist.

In addition a dedicated One Health-symposium on 'Emerging vector born zoonotic parasitoses' will be held. The scientific programme will start right after lunch on Wednesday the 21st of March and we plan to have the last scientific sessions Friday afternoon. Furthermore, several satellite workshops will be organised for converence attendees on Saturday 24th of March.

Dahlem, with its numerous renowned science institutions including four Max-Plank-Institutes, as well as its parks and splendid architecture offers a uniquely sophisticated environment for the meeting. Berlin is certainly an extraordinary venue for meetings of all kinds and provides abundant opportunities for a thrilling post-conference weekend with a unique spectrum of cultural and night-life activities.

As the chairman of the 28th Annual Meeting of the German Society for Parasitology, I would like to thank the DFG for the support to invite international speakers.

Furthermore, I would like to thank all sponsors and exhibitors, especially our main sponsors Bayer Animal Health GmbH, Intervet Deutschland GmbH and Boehringer Ingelheim Vetmedica GmbH very much for their generous support!

Together with my colleagues, I'm looking forward to welcoming you in Berlin at an interesting and stimulating conference with lots of opportunities to discuss new developments, but also to meet old and make new friends.

With best regards

Georg von Samson-Himmelstjerna
Conference chair

PROGRAMME OVERVIEW

Wednesday, 21 March

Lecture hall Audimax	Lecture hall A	Lecture hall B	Lecture hall C	Lecture hall D	Foyer basement
12:30–12:50 **Welcome address**					
12:50–13:50 **Plenary session I** p. 20					
13:50–15:20 **Session I** Molecular genetics p. 20	13:50–15:20 **Session II** Parasitic helminths p. 21	13:50–14:50 **Workshop** Successful publishing and grant writing in parasitology p. 21	13:50–15:20 **Session III** Diagnosis & prevention: phylogeny and evolution p. 22	13:50–15:20 **Session IV** Cells biology and signalling & host parasite interaction p. 22	
Coffee break and industrial exhibition					
15:50–17:30 **Session V** Epidemiology and emerging infections p. 23	15:50–17:30 **Session VI** Vectors, entomology and acarology I p. 24	15:50–17:30 **DDD Symposium** p. 25	15:50–17:30 **Session VII** Veterinary parasitology p. 26		
17:30–18:30 **Award ceremony** p. 26					
					18:30–20:00 **Welcome reception** p. 19

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Session VIII Immunology I p. 28	**Session IX** Protozoan parasites I p. 29	**Workshop** GRK 2046 Parasite-microbiota interaction p. 30	**Session X** Cell biology and signaling II p. 30		
Coffee break and industrial exhibition					
11:00–12:30					
Plenary session II p. 31					
Lunch break and industrial exhibition					
13:30–15:30	13:30–15:30	13:30–15:30	13:30–15:30		
Session XI Immunology II p. 32	**Session XII** Parasite-host interactions I p. 33	**Focus session** Physics of parasitism p. 34	**Session XIII** Drug resistance p. 34		
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DGP board meeting p. 35				**Poster session I** p. 35	
17:00–18:00					
General assembly of the DGP p. 35					

Key

Ceremony/Meeting	Poster session	Social programme
Opening/Closing	Session	Symposium/Workshop
Plenary session	Breaks	

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PROGRAMME OVERVIEW

Friday, 23 March

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Workshop Tungiasis p. 36	**Session XIV** Protozoan parasites II p. 36	**Session XVI** Vectors, entomology and acarology II p. 37	**Session XV** Biochemistry p. 38	
Coffee break and industrial exhibition				
10:30–12:30				
Symposium Emerging vector-born parasitic zoonoses p. 39				
Lunch break and industrial exhibition				12:30–14.00 **Poster session II** p. 40
14:00–15:00				
Plenary session III p. 40				
Coffee break and industrial exhibition				
15:30–17:00	15:30–17:00	15:30–17:00	15:30–17:00	
Focus session Compartments of intracellular parasites p. 40	**Session XVII** Parasite-host interactions II p. 41	**Session XIX** Biochemistry and molecular genetics p. 42	**Session XVIII** Parasite ecology p. 43	
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Institute Par. and Trop. Vet. Med. Room 1	Institute Par. and Trop. Vet. Med. Room 2
09:00–16:00	09:00–12:00
Satellite workshop I AVIAGIS p. 44	**Satellite workshop II** Artificial tick feeding p. 44

Key

Ceremony/Meeting	Poster session	Social programme
Opening/Closing	Session	Symposium/Workshop
Plenary session	Breaks	

GENERAL INFORMATION

Catering
Catering will be served during the official coffee and lunch breaks and is covered by the registration fee.

General certificate of attendance
Certificates of attendance will be available on the last day of the conference at the check-in desk.

Certification and education credits
The 28th Annual Meeting of the German Society for Parasitology (DGP) is certified:

by the 'Akademie für tierärztliche Fortbildung (ATF)'

and

by the 'Ärztekammer Berlin'.

For certification all attendees interested in receiving the hours/points are asked to enter their barcode at the certification station nearby the Check-In.

Cloakroom
A cloakroom is located next to the Check-In desk. The organising agency assumes no liability outside the opening hours. Fee: 1 EUR per garment or piece of luggage.

Conference language
The official congress language is English. The General Assembly of the DGP will be held in German.

Free Wi-Fi access
The Henry Ford Building will provide a free Wifi access.

Login data
Net conference
Code pr8d7anp

General terms and conditions
Please find our general terms and conditions at www.parasitology-meeting.de.

General note

In the following programme, only the first author is cited in oral and poster presentation for reasons of clarity and to save print space. You can find the complete citation in the abstract part.

Name badge

Please wear your name badge during all conference events, including the welcome reception and conference dinner. Admission to scientific sessions, the industrial exhibition as well as the social evening is restricted to participants wearing their badge.

Opening hours

	Wednesday	Thursday	Friday
Check-In	11:30–19:00	08:00–17:00	08:00–18:00
Media Check-In	11:00–17:00	08:00–16:00	08:00–17:00
Industrial exhibition	12:00–20:00	09:30–17:00	09:30–15:45

Presentation and poster awards

The best three oral and poster presentations will be awarded a prize money of 300 EUR, 200 EUR or 100 EUR. The award ceremony will be held on **Friday, 23 March 2018** during the closing remarks.

Registration fees

Student member DPG	125 EUR
Student non-member	145 EUR
Regular member DPG	260 EUR
Regular non-member	300 EUR
Welcome reception, 21 March	inclusive
Social evening, 22 March	45 EUR (regular) 20 EUR (student)

GENERAL TIPS FOR AUTHORS AND PRESENTERS

Presentation upload

The Media Check-In for uploading your presentation is located in the foyer area just outside the plenary hall, nearby the Check-In.
For submission, please use a USB flash drive, CD or DVD disc that is not protected by any software. Professional staff and equipment will be available for you to arrange and preview your presentation.

Submitting your presentation/technical information

The presentation should be prepared as PDF, MS Office PowerPoint for Windows or key for Macintosh DVD in format 4:3.
A presentation notebook with a PDF reader and MS Office PowerPoint 2016 will be provided. The use of personal notebooks is possible upon agreement. However, it may interrupt the flow of the program in the lecture hall. Please provide an adapter for VGA if necessary. To guarantee a smooth running program please upload your presentation on time – at least 2 hours before your presentation starts.

Poster sessions

You can find your permanent programme ID in the programme part of this book. Every poster wall is marked with a permanent programme ID, so that authors can find their place within the poster exhibition.
All poster authors are asked to hang up their posters **on Wednesday, 21 March from 13:00** and **remove them on Friday, 23 March until 15:30**.
Mounting materials will be provided on each poster board. Please do not use any other type of pins than those provided.

Poster authors with an **even programme-ID:**
Please be present in **Poster session I** on Thursday, 22 March l 15:30–17:00.

Poster authors with an **odd programme-ID:**
Please be present in **Poster session II on** Friday, 23 March l 12:30–14:00.

Time allotment

Please prepare your presentation for the allotted amount of time. Chairs may interrupt, should you overrun your time limit. Speaking time is assigned as follows (speaking + discussion time):

Invited speakers	25+5 minutes
Speakers selected from abstracts	12+3 minutes

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Boehringer Ingelheim Vetmedica GmbH (Ingelheim am Rhein/DE)

Check Diagnostics GmbH (Westerau/DE)

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ESCCAP (European Scientific Counsel Companion Animal Parasites) Deutschland e. V. (Köln/DE)

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Zymo Research Europe GmbH (Freiburg/DE)

Transparency

The member companies of the "Voluntary Self-Regulation of the Pharmaceutical Industry (FSA) eV" have more narrowly defined the FSA code to ensure more transparency.

In future, congress organisers are obliged to inform potential congress participants, in advance, about the scope and terms of the support of the pharmaceutical industry. We fulfil this obligation and therefore inform you about the amount of the sponsorship of companies involved:

Bayer Animal Health GmbH: 5.000 EUR (Exhibition booth; Advertisement in the programme; Sponsor of pens and writing pads; Sponsor of lanyards; Sponsor of conference bags; Inlay in the conference bags).

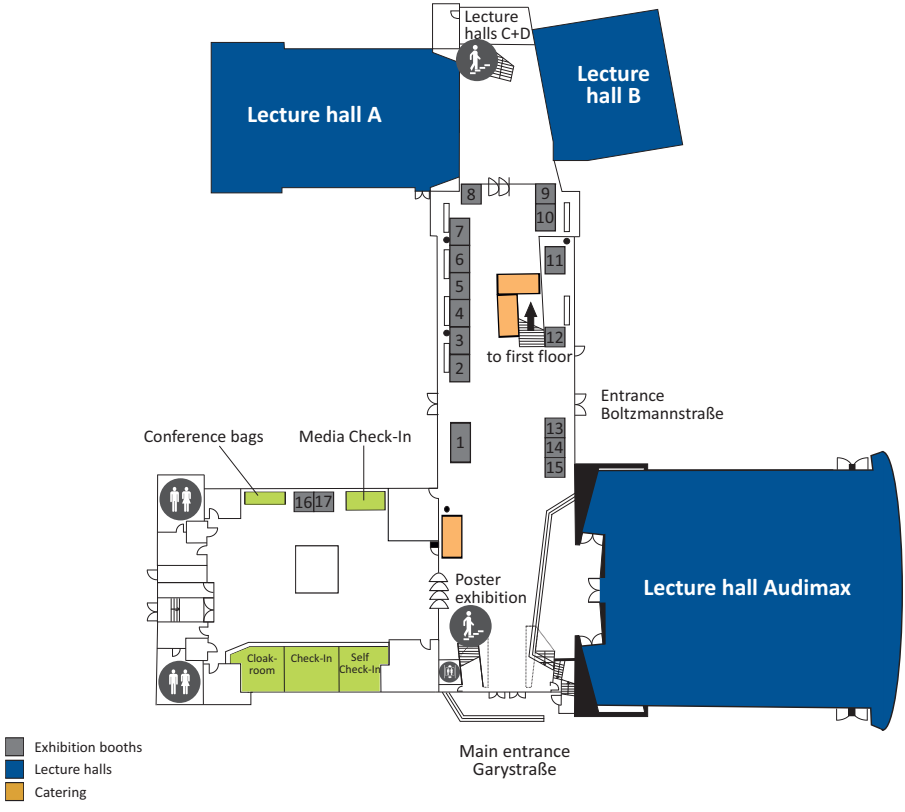
Media cooperations

Cambridge University Press (Cambridge/GB)
"Parasitology"

Trillium GmbH (Grafrath/DE)
"Trillium Diagnostik"

State at printing

PLAN OF THE EXHIBITION AREA



Poster at the second floor and galery

1 Bayer Animal Health GmbH
2 Boehringer Ingelheim Vetmedica GmbH
3 Intervet Deutschland GmbH (MSD)
4 Axon Lab AG
5 Check Diagnostics GmbH
6 GET FIT Massageservice
7 New England Biolabs GmbH
8 Elanco Deutschland GmbH
9 EUROIMMUN AG
10 SARSTEDT AG & Co. KG
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12 Meridian Bioscience Europe
13 Nikon GmbH
14 Springer Spektrum
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16 IDEXX GmbH
17 Wiley-VCH Verlag GmbH & Co. KGaA

State at printing

Welcome reception

Enjoy the first evening with your colleagues and other delegates full of networking possibilities with snacks and refreshments.

Date	21st March
Time	18:30–20:00
Location	venue foyer
Fee	inclusive

Social evening

We would like to invite you to join a memorable social evening at the Fischerhütte Berlin. The location offers a beautiful flair right next to the Schlachtensee. Enjoy a delicious menu in a relaxing atmosphere with your colleagues and friends.

A shuttle will bring you directly from the venue to the location.

Date	22nd March
Time	19:00–24:00
Shuttle	18:30 from venue
Location	Fischerhütte am Schlachtensee I Fischerhüttenstraße 136 I 14163 Berlin
Fee	45 EUR – regular 20 EUR – student
Catering	drinks included until 22:00

12:30–12:50 Opening ceremony • Welcome address
Lecture hall Audimax

12:50–13:50 Plenary session I
Lecture hall Audimax
Chair Georg von Samson-Himmelstjerna (Berlin/DE)

12:50
PL-O-01 Symbiosis and parasitism in filarial nematodes and their *Wolbachia endobacteria*
Mark Blaxter (Edinburgh/GB)

13:20
PL-O-02 MicroRNAs in nematode drug resistance and host-parasite interactions
Collette Britton (Glasgow/GB)

13:50–15:20 Session I • Molecular genetics
Lecture hall Audimax
Chairs Mirko Singer (München/DE), Joachim Clos (Hamburg/DE)

13:50
MOL-O-01 Analysis of individual histone modifications using CRISPR-Cas9
Ines Subota (Munich/DE)

14:05
MOL-O-02 Comparative analysis of different gene editing techniques in *Leishmania donovani*
Henner Zirpel (Hamburg/DE)

14:20
MOL-O-03 Histone acetylation and histone variants during transcription initiation in *Trypanosoma brucei*
Amelie J. Kraus (Munich/DE)

14:35
MOL-O-04 Ribosome Profiling Reveals the Role of HSP90 in Stage-specific Protein Synthesis in *Leishmania donovani*
Joachim Clos (Hamburg/DE)

14:50
MOL-O-05 Six PX domains of Giardia lamblia exhibit diverse phosphoinositide-binding profiles
Ananya Jana (Kolkata/IN)

15:05
MOL-O-06 Minimal ESCRT machinery of Giardia lamblia
Nabanita Saha (Kolkata/IN)

13:50–15:20 Session II • Parasitic helminths

Lecture hall A

Chairs Christina Strube (Hanover/DE), Esra Yilmaz (Berlin/DE)

13:50
PAH-O-01 Genome wide expression profiling of the *Echinococcus multilocularis* stem cell system
Michaela Herz (Würzburg/DE)

14:05
PAH-O-02 Exploratory metabolomics study of the experimental opisthorchiasis in a laboratory animal model (golden hamster, Mesocricetus auratus)
Oleg Mayboroda (Leiden/NL)

14:20
PAH-O-03 Forecasts of attaining onchocerciasis elimination in Ogun State, Nigeria: a cross-sectional report of the Ov-16 serology (Rapid Diagnostic Test and ELISA) among children born after 10 years of treatment with ivermectin
Olabanji Surakat (Abeokuta/NG)

14:35
PAH-O-04 Histopathological changes during the course of *Toxocara canis*- and *T. cati*-induced neurotoxocarosis
Andrea Springer (Hanover/DE)

14:50
PAH-O-05 Concurrent helminth infection interferes with vaccine-induced protection against influenza virus infection
Minka Breloer (Hamburg/DE)

15:05
PAH-O-06 Host-parasite-microbiota interactions in thoroughbred horses
Laura Peachey (Cambridge/GB)

13:50–14:50 Workshop • Successful publishing and grant writing in parasitology

Lecture hall B

13:50 tba
Andreas Strecker (Bonn/DE)

13:50–15:20 Lecture hall C	Session III • Diagnosis & prevention: phylogeny and evolution
Chairs	Ralf Ignatius, Karsten Nöckler (Berlin/DE)
13:50 DPP-O-01	Establishing a protocol for simultaneous comparative molecular and proteomic species identification of individual cyathostomin worms Christina Bredtmann (Berlin/DE)
14:05 DPP-O-02	The application of isothermal nucleic acid amplification in diagnostics and point-of-care testing for the detection of parasites Sebastian Kersting (Potsdam/DE)
14:20 DPP-O-03	Pre-clinical evaluation of a double genetically arrested parasite (dKO GAP) for malaria vaccine development Oriana Kreutzfeld (Berlin/DE)
14:35 DPP-O-04	Onchocerciasis Associated Epilepsy is preventable – case of Bilomo in the Mbam Valley, Cameroon Joseph Nelson Siewe Fodjo (Antwerp/BE)
14:50 DPP-O-06	Phylogeny and phylogeography of *echinococcus granulosus* genotypes G6-G10 based on complete mitochondrial genomes and six nuclear loci Teivi Laurimäe (Tartu/EE)

13:50–15:20 Lecture hall D	Session IV • Cells biology and signalling & host parasite interaction
Chairs	Dennis Klug (Strasbourg/FR), Suzana Zakovic (Berlin/DE)
13:50 CBH-O-01	Co-transcriptional nuclear export of mRNAs in trypanosomes Susanne Kramer (Würzburg/DE)
14:05 CBH-O-02	Good MORNing – new insights into the structure and function of a MORN repeat protein from *Trypanosoma brucei* Brooke Morriswood (Würzburg/DE)
14:20 CBH-O-03	Patatin-like phospholipases of the human malaria parasite *Plasmodium falciparum* Ansgar Flammersfeld (Aachen/DE)

14:35 CBH-O-04	An exclusive guanylate cyclase governs the lytic cycle of *Toxoplasma gondii* Ozlem Günay-Esiyok (Berlin/DE)
14:50 CBH-O-05	Binding of host C-type lectin receptors to *Toxocara* spp.-derived ligands Marie-Kristin Raulf (Hanover/DE)
15:05 CBH-O-06	Starving a deadly parasite – *in vitro* metabolomic studies on the *Echinococcus multilocularis* metacestode Dominic Ritler (Bern/CH)

15:50–17:30 Session V • Epidemiology and emerging infections

Lecture hall Audimax

Chairs: Cornelia Silaghi (Greifswald/DE), Julia Walochnik (Vienna/AT)

15:50 EPI-O-01	Spread of human dirofilariasis in Europe Renke Lühken (Hamburg/DE)
16:05 EPI-O-02	Molecular identification of tick-borne pathogens infecting cattle in Asia and Africa reveals emerging Anaplasma, Ehrlichia and Babesia species Ard Nijhof (Berlin/DE)
16:20 EPI-O-03	Prevalence of metastrongyloid lungworm larvae in Colombian giant African snails (*Achatina fulica*) Malin Katharina Lange (Giessen/DE)
16:35 EPI-O-04	Epidemiology of *Acanthamoeba* infections Julia Walochnik (Vienna/AT)
16:50 EPI-O-05	Leishmaniasis in Uzbekistan – an epidemiological update Katrin Kuhls (Wildau/DE)
17:05 EPI-O-06	Emerging foci of visceral leishmaniasis in Armenia – molecular epidemiology and pilot risk assessment by ecological niche modeling Katrin Kuhls (Wildau/DE)
17:20 EPI-O-07	Investigation of causative agent of cat scratch disease in cats and their fleas in Lithuania Indre Lipatova (Kaunas/LT)

15:50–17:20 Lecture hall A	Session VI • Vectors, entomology and acarology I
Chairs	Peter-Henning Clausen (Berlin/DE), Stefanie Becker (Hanover/DE)
15:50 VEA-O-01	*Culex torrentium* mosquitoes from Germany are negative for *Wolbachia* Stefanie Christine Becker (Hamburg, Hanover/DE)
16:05 VEA-O-02	Mosquito-based survey of filarial nematodes circulating in Germany Mandy Schäfer (Greifswald – Insel Riems/DE)
16:20 VEA-O-03	A comparative analysis of subsampling methods to estimate the number of specimens and species in large mosquito samples Linda Jaworski (Hamburg, Oldenburg/DE)
16:35 VEA-O-04	Can *onchocerciasis* be eradicated? Epidemiological studies in three bio-geographic regions of Cameroon and experimental approach in the bovine *Onchocerca ochengi* model Alfons Renz (Tübingen/DE)
16:50 VEA-O-05	The potential for the use of the *Theileria parva* live vaccine cocktail at the wildlife-livestock interface in Uganda – the range of epidemiological situations and antigen gene conservation Isaiah Obara (Berlin/DE)
17:05 VEA-O-06	Ecto- and hemoparasites of two small-bodied Malagasy primate species (*Microcebus murinus* and *M. ravelobensis*) Annette Klein (Hanover/DE)

15:50–17:30	DDD Symposium
Lecture hall B	
Chairs	Claudia Welz (Köln/DE), Paul Selzer (Tübingen/DE)
15:50 DDD-O-01	Evaluation of the Pharmacokinetic-Pharmacodynamic Relationship of Praziquantel in the *Schistosoma mansoni* Mouse Model Thomas Spangenberg (Coinsins/CH)
16:05 DDD-O-02	Effect of arthropod antimicrobial peptides against *Plasmodium falciparum* Miray Tonk (Giessen/DE)
16:20 DDD-O-03	Alive and green – Refining Chagas' disease in vitro drug screening Anna F. Fesser (Basel/CH)
16:35 DDD-O-04	*Echinococcus multilocularis* – from drug screenings to the energy metabolism Reto Rufener (Bern/CH)
16:50 DDD-O-05	Pharmacokinetics, efficacy and safety of ascending dosages of ivermectin against *Trichuris trichiura* in preschool- and school-aged children Jessica D. Schulz (Basel/CH)
17:05 DDD-O-06	Insights on the mode of action of the novel isoxazoline ectoparasiticide Heinz Sager (Basel/CH)
17:20 DDD-O-07	Ladybird beetles and schistosomes – inhibitory effects of the novel antimicrobial compound harmonine on survival and reproduction of *Schistosoma mansoni* Simone Häberlein (Giessen/DE)

15:50–17:30 Lecture hall C	Session VII • Veterinary parasitology
Chairs	Anja Joachim (Vienna/AT), Zaida Renteria (Leipzig/DE)
15:50 VPA-O-01	The impact of patent *Fasciola hepatica* infections on individual milk production and fertility parameters in dairy cows Katharina May (Hanover, Giessen/DE)
16:05 VPA-O-02	Long-term monitoring of changes in endoparasitic infections as a result of pasture re-wetting in livestock in Northern Germany Katrin Blazejak (Hanover/DE)
16:20 VPA-O-03	*Coccidiosis* and other parasitosis in milk livestocks from Lower Saxony: effects of different husbandry systems Nele Loock (Berlin/DE)
16:35 VPA-O-04	Genetic and transcriptomic characterization of the P-glycoprotein gene family in *Parascaris sp.* Alexander Gerhard (Berlin/DE)
16:50 VPA-O-05	Pathological findings in intestines of grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) from the North and Baltic Seas associated with acanthocephalan infections Jan Lakemeyer (Büsum/DE)
17:05 VPA-O-06	Reaction of immune cells within the intestine of thinlip mullet, Liza ramada (Pisces), in response to micro- and macro-parasitic infection Bahram Sayyaf Dezfuli (Ferrara/IT)
17:20 VPA-O-07	Involvement of rodlet cells in fish organs infected with metazoan parasites Bahram Sayyaf Dezfuli (Ferrara/IT)

17:30–18:30 Lecture hall Audimax	Award ceremony Piekarski-Preis Karl Asmund Rudolphi-Medaille Rudolf Leuckart-Medaille

18:30–20:00 Foyer basement	Welcome reception (see page 19)

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STRONGHOLD® PLUS 15 mg/2,5 mg Lösung zum Auftropfen für Katzen **STRONGHOLD® PLUS** 30 mg/5 mg Lösung zum Auftropfen für Katzen **STRONGHOLD® PLUS** 60 mg/10 mg Lösung zum Auftropfen für Katzen **WIRKSTOFFE UND SONSTIGE BESTANDTEILE:** Jede Einzeldosis (Pipette) enthält: Wirkstoff(e): **STRONGHOLD® PLUS** Lösung zum Auftropfen für Katzen (≤2,5 kg / >2,5–5 kg / >5–10 kg): Pipetteninhalt (0,25 ml / 0,5 ml / 1 ml): Selamectin (15 mg / 30 mg / 60 mg); Sarolaner (2,5 mg / 5 mg / 10 mg). Sonstige Bestandteile: Butylhydroxytoluol. Dipropylenglykolmethylether. Isopropylalkohol. **ANWENDUNGSGEBIET(E):** Für Katzen, bei denen eine parasitäre Mischinfektion durch Zecken, Flöhe, Läuse, Milben, gastrointestinale Nematoden oder Herzwürmer vorliegt, oder das Risiko einer solchen parasitären Mischinfektion besteht. Die Anwendung des Tierarzneimittels ist ausschließlich für eine gleichzeitige Behandlung gegen Zecken und mindestens einen der anderen genannten Zielparasiten angezeigt. Ektoparasiten: • Zur Behandlung und Vorbeugung von Flohbefall (*Ctenocephalides* spp.). Das Tierarzneimittel hat eine sofortige und anhaltende Floh tötende Wirkung für 5 Wochen gegen neuen Flohbefall. Es tötet 5 Wochen lang adulte Flöhe, bevor diese Eier legen. Durch seine ovizide und larvizide Wirkung kann das Tierarzneimittel zur Kontrolle von bestehendem Flohbefall in Bereichen beitragen, zu denen das Tier Zugang hat. • Das Tierarzneimittel kann als Teil der Behandlungsstrategie bei allergischer Flohdermatitis (FAD) verwendet werden. • Behandlung von Zeckenbefall. Das Tierarzneimittel hat eine sofortige und anhaltende akarizide Wirkung für 5 Wochen gegen *Ixodes ricinus* und *Ixodes hexagonus*, sowie für 4 Wochen gegen *Dermacentor reticulatus* und *Rhipicephalus sanguineus*. • Behandlung von Ohrmilben (*Otodectes cynotis*). • Behandlung von Läusebefall (*Felicola subrostratus*). Zecken müssen am Wirt anheften und mit der Nahrungsaufnahme begonnen haben, um dem Wirkstoff Sarolaner ausgesetzt zu werden. Nematoden: • Behandlung von adulten Spulwürmern (*Toxocara cati*) und adulten intestinalen Hakenwürmern (*Ancylostoma tubaeforme*). • Vorbeugung von Herzwurmerkrankungen, verursacht durch *Dirofilaria immitis*, bei monatlicher Verabreichung. **GEGENANZEIGEN:** Nicht anwenden bei Katzen, die gleichzeitig an einer anderen Erkrankung leiden oder die geschwächt und untergewichtig (in Größe und Alter) sind. Nicht anwenden bei Überempfindlichkeit gegenüber dem Wirkstoff oder einem der sonstigen Bestandteile. **NEBENWIRKUNGEN:** Die Anwendung des Tierarzneimittels kann zu einem leichten, vorübergehenden Juckreiz am Auftragungsort führen. Gelegentlich wurden leichter bis mittelgradiger Haarausfall am Ort der Anwendung, Rötung und Speicheln beobachtet. Besondere Warnhinweise für jede Zieltierart: Im Einklang mit der guten Veterinärpraxis wird empfohlen, alle Tiere im Alter von 6 Monaten oder mehr, die in Ländern mit einem vorhandenen Vektor leben, vor Beginn der präventiven Anwendung des Tierarzneimittels auf bestehende Infektionen mit adulten Herzwürmern untersucht werden. Dieses Tierarzneimittel ist nicht wirksam gegen adulte *D. immitis*. Die Anwendung bei Tieren, die an Infektionen mit adulten Herzwürmen erkrankt waren, warf keine Sicherheitsbedenken auf. Obwohl nicht routinemäßig indiziert, sollten die Vorteile von regelmäßig durchgeführten Kontrolluntersuchungen auf Herzwurmerkrankungen in Einzelfällen vom behandelnden Tierarzt berücksichtigt werden. Zecken müssen mit der Nahrungsaufnahme auf dem Wirt begonnen haben, um dem Wirkstoff Sarolaner ausgesetzt zu werden. Daher kann eine Übertragung von infektiösen Krankheiten durch Zecken nicht ausgeschlossen werden. **WARTEZEIT:** Nicht zutreffend. DE: Verschreibungspflichtig. AT: Rezept- und apothekenpflichtig. Zulassungsinhaber: Zoetis Belgium S.A., Rue Laid Burniat 1, 1348 Louvain-La-Neuve, Belgien. Örtlicher Vertreter DE: Zoetis Deutschland GmbH, Schellingstraße 1, 10785 Berlin. Örtlicher Vertreter AT: Zoetis Österreich GmbH, Floridsdorfer Hauptstraße 1, A - 1210 Wien.

zoetis

08:30–10:30 Session VIII • Immunology I

Lecture hall Audimax

Chairs Friederike Ebner (Berlin/DE), Marc Hübner (Bonn/DE)

08:30
IMM-O-01 The outcome of *Entamoeba* histolytica-induced liver damage is influenced by sex-dependent activation of macrophages and monocytes
Julie Sellau (Hamburg/DE)

08:45
IMM-O-02 Intestinal oncosphere invasion of *Echinococcus multilocularis*: Investigation of resistance mechanisms in a new rat model
Deborah Joekel (Zürich/CH)

09:00
IMM-O-03 Site-specific effects of IL-33 treatment during helminth infection: increased parasite burden in the tissue and reduced parasite burden in the intestine
Martina Reitz (Hamburg/DE)

09:15
IMM-O-04 Dry season *P. falciparum* asymptomatic infections' impact on the host immunity
Carolina M. Andrade (Heidelberg/DE)

09:30
IMM-O-05 Molecular mechanisms of bovine NET formation induced by *Toxoplasma gondii* tachyzoites
Iván Conejeros (Giessen/DE)

09:45
IMM-O-06 *Dirofilaria immitis* microfilariae and third stage larvae induce different types of NETs in canine PMN
Tamara Muñoz-Caro (Giessen/DE)

10:00
IMM-O-07 S100A9 knockout increases inflammatory immune responses upon *L. sigmodontis* L3 larvae and impairs larval migration
Stefan Frohberger (Bonn/DE)

10:15
IMM-O-08 Filarial cystatin induced immunomodulation in human monocytes and macrophages
Gopinath Venugopal (Berlin/DE)

08:30–10:30 Lecture hall A	Session IX • Protozoan parasites I
Chairs	Kathrin Buchholz (Giessen/DE), Florence Awamu Ndonglack (Berlin/DE)
08:30 PRO-O-01	Dissecting the expression, localization and function of the *P. falciparum* STEVOR family Jan Stephan Wichers (Hamburg/DE)
08:45 PRO-O-02	Towards understanding epigenetic gene regulation of differentiating male and female *P. falciparum* gametocytes Michaela Petter (Erlangen/DE; Melbourne/AU)
09:00 PRO-O-03	Complement factor H-related protein 1 impairs factor H acquisition during complement evasion the by malaria parasite *Plasmodium falciparum* Timo Reiß (Aachen/DE)
09:15 PRO-O-04	The role of *Plasmodium falciparum* derived microvesicles in malaria related anemia Florence Awamu Ndonglack (Berlin/DE)
09:30 PRO-O-05	*P. falciparum* glycans in adaptive immune recognition and their vaccine potential Jonnel Anthony Jaurigue (Berlin/DE)
09:45 PRO-O-06	Epidemiological aspects of Phlebotomine sand fly species, *Leishmania* vectors, from Old and New World – Alentejo region, Southern Portugal, and Volta Redonda Municipality, Brazil, 2016/2017 Sara Pereira (Lisbon/PT)
10:00 PRO-O-07	*Toxoplasma gondii* infections in chickens – performance of various antibody detection techniques in serum and meat juice relative to bioassay and DNA detection methods Gereon Schares (Greifswald – Insel Riems/DE)
10:15 PRO-O-08	Use of CRISPR/cas 9 to establish transgenic *Cryptosporidium parvum* strain *in vitro* Wanpeng Zheng (Leipzig/DE)

08:30–10:30 GRK 2046 Workshop • Parasite-microbiota interaction
Lecture hall B
Chairs Ivet Yordanova, Totta Ehret (Berlin/DE)

08:30 WSG-O-01 Of worms, germs and men – from nature to the clinic
Cinzia Cantacessi (Cambridge/GB)

09:00 WSG-O-02 Secreted products of the intestinal roundworm *Ascaris suum* impact bacterial growth and biofilm formation
Ankur Midha (Berlin/DE)

09:15 WSG-O-03 Gut microbial changes in mice experimentally infected with *Schistosoma mansoni*
Timothy Jenkins (Cambridge/GB)

09:30 WSG-O-04 Metabolism and early encystation of high-density *Giardia* foci in the mouse small intestine
Scott C. Dawson (Davis, CA/US)

10:00 WSG-O-05 Role of the REL2/Imd signaling pathway and the host-microbes interplay in the vectorial capacity of *Anopheles gambiae*
Suzana Zakovic (Berlin/DE)

10:15 WSG-O-06 Metabarcoding the bacterial and eukaryotic (micro-)biome reveals an abundance of unknown intestinal inhabitants
Emanuel Heitlinger (Berlin/DE)

08:30–10:30 Session X • Cell biology and signaling
Lecture hall C
Chairs Christoph G. Grevelding (Gießen/DE), Oriana Kreuzfeld (Berlin/DE)

08:30 CBS-O-01 A ligand switch in the unusual *Trypanosoma* Protein Kinase A suggests a novel second messenger pathway
Michael Boshart (Martinsried/DE)

08:45 CBS-O-02 Molecular and physiological analysis of social behaviour in *Trypanosoma brucei*
Sabine Bachmaier (Martinsried/DE)

09:00
CBS-O-03
Impact of HSP90 Phosphorylation on the Life Cycle Stages of *Leishmania donovani*
Joachim Clos (Hamburg/DE)

09:15
CBS-O-04
Towards understanding how *Plasmodium Sporozoites* are formed
Freddy Frischknecht (Heidelberg/DE)

09:30
CBS-O-05
A SMYD3 Histone-Lysine N-Methyltransferase Homologue Regulates Life Cycle Progression in *Plasmodium Parasite*
Sebastian Kirchner (Glasgow/GB)

09:45
CBS-O-06
Deciphering the regulation of the mayor S-phase promoting factor *Plasmodium falciparum* CRK4
Markus Ganter (Heidelberg/DE)

10:00
CBS-O-07
Cooperative integrin/SmVKR-1 signaling controls cell survival in the ovary of paired *Schistosoma mansoni* females
Christoph G. Grevelding (Giessen/DE)

10:15
CBS-O-08
A functional wnt signaling pathway is essential for *Echinococcus multilocularis* larval development
Klaus Brehm (Würzburg/DE)

11:00–12:30 Plenary session II

Lecture hall Audimax

Chair: Kai Matuschewski (Berlin/DE)

11:00
PL-O-03
Regulation of adaptive immunity during *Plasmodium* infection
Wiebke Hansen (Essen/DE)

11:30
PL-O-04
The complexity and the simplicity of host-*Plasmodium* interactions
Maria Manuel Dias da Mota (Lisboa/PT)

12:00
PL-O-05
Dirofilaria as a model for vector-pathogen-interactions – vector competence and transcriptomic analysis
Cornelia Silaghi (Greifswald/DE)

13:30–15:30	Session XI • Immunology II
Lecture hall Audimax	
Chairs	Wiebke Hartmann (Hamburg/DE), Sebastian Rausch (Berlin/DE)
13:30 IMM-O-09	Targeting *Ascaris* – specific CD4+ T-cells and MHC-restricted T-cell epitopes of *Ascaris* antigens Friederike Ebner (Berlin/DE)
13:45 IMM-O-10	Helminth-induced interference with vaccination efficacy and the role of type 1 regulatory T-cells Wiebke Hartmann (Hamburg/DE)
14:00 IMM-O-11	Protozoan co-infection leads to a Th1 immune response in helminth-specific T-cells Norus Ahmed (Berlin/DE)
14:15 IMM-O-12	Dendritic cell-specific OTUB1 is crucial for a strong and effective immune response against *Toxoplasma gondii* infection Floriana Mulas (Magdeburg/DE)
14:30 IMM-O-13	Identification of novel receptors contributing to LC3-associated phagocytosis in *Leishmania* infection Ger van Zandbergen (Langen, Mainz/DE)
14:45 IMM-O-14	Balancing Th2 immunity – Th2/1 hybrid cells in parasite infections and atopy Sebastian Rausch (Berlin/DE)
15:00 IMM-O-15	Schistosome egg antigens, including the glycoprotein IPSE/alpha-1, trigger the development of regulatory B cells Simone Häberlein (Giessen/DE; Leiden/NL)
15:15 IMM-O-16	Host determinants of susceptibility to *Giardia* infection Ivet Yordanova (Berlin/DE)

13:30–15:30 Lecture hall A	Session XII • Parasite-host interactions I
Chairs	Francesca Torelli (Berlin/DE), Alyssa Ingmundson (Berlin/DE)
13:30 PHI-O-01	*Giardia duodenalis* infection of human intestinal cells – modelling asymptomatic colonization? Martin Kraft (Berlin/DE)
13:45 PHI-O-02	*Trypanosoma cruzi* infection mediates phosphoproteomic networks associated with pathology in colonic epithelial cells Pius Nde (Nashville, TN/US)
14:00 PHI-O-03	African Trypanosomes Can Evade Immune Clearance by Sugar-Coating Antigenic Surfaces Francisco Aresta Branco (Heidelberg/DE)
14:15 PHI-O-04	Impact of naturally acquired immunity to *Plasmodium falciparum* on var gene expression in controlled human malaria infections Anna Bachmann (Hamburg/DE)
14:30 PHI-O-05	Changes in the transcriptome of human brain endothelial cells (HBECs) in response to *Plasmodium falciparum* malaria infection Michael Dörpinghaus (Hamburg/DE)
14:45 PHI-O-06	Association of TNF-α -308G/A polymorphism with *Plasmodium falciparum* and *Schistosoma haematobium* infections in Nigerian children Olusola Ojurongbe (Osogbo/NG)
15:00 PHI-O-07	A cell culture-based system to assess the role of Immunity-Related GTPases in the maintenance of virulent *Toxoplasma gondii* strains in wild rodents Francesca Torelli (Berlin/DE)
15:15 PHI-O-08	House mouse hybrids show higher vigour in response to *Eimeria* infection compared to pure (sub-)species Alice Balard (Berlin/DE)

13:30–15:30	Focus session • Physics of parasitism
Lecture hall B	
Chairs	Francisco Aresta Branco (Heidelberg/DE), Markus Engstler (Würzburg/DE)
13:30 POP-O-01	The Physics of Parasitism Markus Engstler (Würzburg/DE)
13:45 POP-O-02	Narrow escape – How long does it take for a camel to go through the eye of a needle? Susanne Fenz (Würzburg/DE)
14:00 POP-O-03	An *in silico* model for the African trypanosome Holger Stark (Berlin/DE)
14:15 POP-O-04	Modeling Malaria Invasion of Red Blood Cells – and Beyond Gerhard Gompper (Jülich/DE)
14:30 POP-O-05	Modelling cytoadhesion of malaria-infected red blood cells Ulrich S. Schwarz (Heidelberg/DE)
14:45 POP-O-06	Understanding force transduction during malaria parasite migration Freddy Frischknecht (Heidelberg/DE)
15:00 POP-O-07	The role of actin in apicomplexan – Back on track? Markus Meissner (Munich/DE)
15:15 POP-O-08	Understanding the physics of hydatid cysts Klaus Brehm (Würzburg/DE)

13:30–15:30	Session XIII • Drug resistance
Lecture hall C	
Chairs	Jürgen Krücken (Berlin/DE), Laura Peachy (Cambridge/GB)
13:30 DRE-O-01	Predicting drug resistance evolution Jens Rolff (Berlin/DE)
14:00 DRE-O-02	Macrocyclic lactones – activation of a new subtype of glutamate-gated chloride channels in *Parascaris sp.* Nicolas Lamassiaude (Nouzilly/FR)

14:15 DRE-O-03	Cytochrome P450 expression in benzimidazole susceptible- and resistant isolates of *Haemonchus contortus* following exposure to thiabendazole Esra Yilmaz (Berlin/DE)
14:30 DRE-O-04	Genetic basis of benzimidazole resistance in *Caenorhabditis elegans* wild isolates Steffen Hahnel (Evanston, IL/US)
14:45 DRE-O-05	Occurrence of insecticide resistance in stable flies (*Stomoxys calcitrans*) on dairy farms in the federal state of Brandenburg, Germany Sophia Reissert (Berlin/DE)
15:00 DRE-O-06	More than immune evasion – a variant surface glycoprotein causes *in vitro* suramin resistance in *Trypanosoma brucei* Natalie Wiedemar (Basel/CH)
15:15 DRE-O-07	Phenotypic characterization of a nitro drug resistant *Giardia lamblia* strain Joachim Müller (Bern/CH)

15:30–17:00 Poster session I
Foyer upstairs
(see page 14)

15:30–17:00 DGP board meeting
Konferenzzimmer 1 (K1)

17:00–18:00 General assembly of the DGP
Lecture hall Audimax

19:00–23:00 Social evening
Fischerhütte
(see page 19)

08:30–10:00 Workshop • Tungiasis

Lecture hall Audimax

Chairs Francis Mutebi (Kampala/UG), Hermann Feldmeier (Berlin/DE)

08:30 WOT-O-O1 *Tunga penetrans* infections among animals and humans in East Africa
Francis Mutebi (Kampala/UG)

09:00 WOT-O-O2 Prevalence, intensity and risk factors of tungiasis in Kilifi County, Kenya: I. Results from a community-based study
Susanne Wiese (Berlin/DE)

09:15 WOT-O-O3 High infection frequency with *Wolbachia pipientis* and potentially transmissible *Rickettsia bellii*-like bacteria in *Tunga penetrans* from Uganda and Kenya
Jürgen Krücken (Berlin/DE)

09:30 WOT-O-O4 Very severe tungiasis – a life-threatening condition
Hermann Feldmeier (Berlin/DE)

09:45 WOT-O-O5 Control of tungiasis – experiences and challenges
Marlene Thielecke (Berlin/DE)

08:30–10:00 Session XIV • Protozoan parasites II

Lecture hall A

Chairs Anja Taubert (Gießen/DE), Jonnel Anthony Jaurigue (Berlin/DE)

08:30 PRO-O-09 Use it or lose it – function and functionality of mitochondrial DNA in the sleeping sickness parasite *Trypanosoma brucei*
Achim Schnaufer (Edinburgh/GB)

08:45 PRO-O-10 The novel telomere-associated protein TelAP1 links stage-specific telomere complexes with developmental expression site silencing in *African trypanosomes*
Christian Janzen (Mainz/DE)

09:00 PRO-O-11 Viral discovery and diversity in trypanosomatids with a focus on relatives of the human parasite *Leishmania*
Vyacheslav Yurchenko (Ostrava/CZ)

09:15
PRO-O-12
Trans-acting GC-rich non-coding RNA at var expression site modulates gene counting in malaria parasite
Julien Guizetti (Heidelberg/DE)

09:30
PRO-O-13
An unusual prohibitin regulates malaria parasite mitochondrial membrane potential
Joachim Michael Matz (Nijmegen/NL)

09:45
PRO-O-14
Profile of cholesterol-related sterols and impact of selected oxysterols in *Eimeria bovis* macromeront formation in bovine host endothelial cells
Liliana M.R. Silva (Giessen/DE)

08:30–10:00 Session XVI • Vectors, entomology and acarology II

Lecture hall B

Chairs: Ard Nijhof (Berlin/DE), Lidia Chitimia-Dobler (München/DE)

08:30
VEA-O-07
Molecular interactions of *Trypanosoma cruzi*, triatomines and intestinal bacteria – a review
Günter Schaub (Bochum/DE)

08:45
VEA-O-08
Multiantennary N-glycans as unique natural glycodendrimers of the canine heartworm
Francesca Martini (Zürich/CH)

09:00
VEA-O-09
Tick abundance in the city of Hanover
Daniela Hauck (Hanover/DE)

09:15
VEA-O-10
Vector role and control of the tick *Dermacentor reticulatus* in Poland
Anna Bajer (Warsaw/PL)

09:30
VEA-O-11
Amblyomma birmitum sp. nov. and *Haemaphysalis cretacea* sp. nov.: two new hard tick species in Burmese amber
Lidia Chitimia-Dobler (Munich/DE)

09:45
VEA-O-12
Tick-borne pathogens detection in African cattle by Reverse Line Blot microarray
Babette Josiane Guimbang Abanda (Ngaoundéré/CM; Tübingen/DE)

08:30–10:00	Session XV • Biochemistry
Lecture hall C	
Chairs	Michael Boshart (Planegg-Martinsried/DE), Nishith Gupta (Berlin/DE)
08:30 BIO-O-01	Comparative Nematode Glycomics Katharina Paschinger (Vienna/AT)
08:45 BIO-O-02	Comparative Trichomonad Glycomics Iain Wilson (Vienna/AT)
09:00 BIO-O-03	Understanding the biological role of parasitic N-glycans using microarrays Barbara Eckmair (Vienna/AT)
09:15 BIO-O-04	Structural analyses of nematode N-glycan post-translational modifications elucidate conserved functional epitopes and biosynthetic pathways Shi Yan (Vienna/AT)
09:30 BIO-P-05	The metabolic role of amylopectin turnover in *Toxoplasma gondii* Martin Blume (Berlin/DE)
09:45 BIO-O-06	*Toxoplasma gondii* harbors a pathway to synthesize amino acids otherwise essential for its mammalian host cells Gupta Nishith (Berlin/DE)

10:30–12:30	Symposium • Emerging vector-born parasitic zoonoses
Lecture hall Audimax	
Chairs	Toni Aebischer, Franz Conraths (Berlin/DE)
10:30 SEP-O-01	Veterinary relevance and potential for spread of dirofilariosis Laura Kramer (Parma/IT)
10:50 SEP-O-02	Emerging Human dirofilariasis as a medical problem Vladimir Kartashev (Rostov-na-Donu/RU)
10:50 SEP-O-03	Clinical management of canine leishmaniosis – towards a One Health approach Guadalupe Miró (Madrid/ES)
11:10 SEP-O-04	Emerging human leishmaniosis in Europe – the need of one health approach Rogelio López-Vélez (Madrid/ES)
11:30	Panel discussion

12:30–14:00 Poster session II
Foyer upstairs
(see page 14)

14:00–15:00 Plenary session III
Lecture hall Audimax
Chair Susanne Hartmann (Berlin/DE)

14:00
PL-O-06
Theileria parva live and subunit vaccines and genomics – an overview
Richard Bishop (Pullman, WA/US)

14:30
Pl-O-07
Parasite diversity and competitor-predator-prey relationships: recolonising grey wolves in central Europe as a "natural experiment"
Heribert Hofer (Berlin/DE)

15:30–17:00 Focus session • Compartments of intracellular parasites
Lecture hall Audimax
Chairs Gabriele Pradel, Teresa Maria Anslinger (Aachen/DE)

15:30
CIP-O-06
A novel *Plasmodium* protein and its critical function for host-to-vector transmission
Klara Vochyanova (Heidelberg/DE)

15:45
CIP-O-01
Understanding gliding motility of the malaria parasite through proteomics approaches
Jessica Kehrer (Heidelberg/DE)

16:00
CIP-O-02
The protozoan *Toxoplasma gondii* uses a multi-functional device that mechanically mimics dynamins to give birth to a unique parasitophorous vacuole prone to rapid remodelling
Isabelle Tardieux (Grenoble la Tronche/FR)

16:15
CIP-O-03
The role of host factors in *P. falciparum*-mediated host erythrocyte modification
Jude Przyborski (Heidelberg/DE)

16:30
CIP-O-04
Unraveling the function of EXP1 in the asexual development of *P. falciparum* parasites
Paolo Mesen Ramirez (Hamburg/DE)

16:45
CIP-O-05
A stress granule-resident seven-helix protein regulates translation during transmission of the malaria parasite *Plasmodium falciparum*
Sandra Bennink (Aachen/DE)

15:30–17:00	Session XVII • Parasite-host interactions II
Lecture hall A	
Chairs	Carsten Lüder (Göttingen/DE), Philipp Olias (Bern/CH)
15:30 PHI-O-14	Multiplex profiling of inflammation-related mediators in *Toxocara canis*- and *T. cati*-infected brains Patrick Waindok (Hanover/DE)
15:45 PHI-O-09	What can we learn about the function of the key *Toxoplasma gondii* virulence effector protein IST in closely related *Hammondia hammondi* and *Neospora caninum*? Philipp Olias (Bern/CH)
16:00 PHI-O-10	Chronic *Toxoplasma gondii* infection leads to phenotypic changes in human monocytes *in vivo* and *in vitro* Carsten Lüder (Göttingen/DE)
16:15 PHI-O-11	Inhibition of inflammasome activation in human cells upon *Toxoplasma gondii* infection Mateo Murillo Leon (Freiburg/DE)
16:30 PHI-O-12	Establishment of *Babesia microti* developmental cycle and its experimental application Marie Jalovecka (České Budějovice/CZ)
16:45 PHI-O-13	Murine eosinophils trap microfilariae of the rodent filarial nematode *Litomosoides sigmodontis* in an ETosis-dependent mechanism Alexandra Ehrens (Bonn/DE)

15:30–17:00 Session XIX • Biochemistry and molecular genetics

Lecture hall B

Chairs Klaus Brehm (Würzburg/DE), Frank Seeber (Berlin/DE)

15:30
BMG-O-01
A synthetic promoter for multi-stage expression reveals complementary functions of *Plasmodium adhesins*
Dennis Klug (Strasbourg/FR)

15:45
BMG-O-02
Interdomain GFP-tagging of the Plasmodium circumsporozoite protein visualizes sporozoite formation and reveals C-terminal processing
Mirko Singer (Heidelberg, Munich/DE)

16:00
BMG-O-03
Gain and loss of small RNA classes in Strongyloididae
Adrian Streit (Tübingen/DE)

16:15
BMG-O-04
The regulatory subunit of a PKA like kinase in Trypanosoma brucei: structural studies for novel tools
Yuri Volpato (Martinsried/DE)

16:30
BMG-O-05
The structure of serum-resistance-associated protein and its implications for Human African Trypanosomiasis
Sebastian Zoll (Oxford/GB)

16:45
BMG-O-06
Comparative transcriptomics of Opisthorchiidae liver flukes: identification of potential therapeutic targets in detoxification system
Mariya Pakharukova (Novosibirsk/RU)

„Gerhard Piekarski-Preis“

15:30–17:00	Session XVIII • Parasite ecology
Lecture hall C	
Chairs	Heribert Hofer, Susana Ferreira (Berlin/DE)
15:30 ECO-O-01	The role of parasites in ecology and evolution Peter Wenk (Tübingen/DE)
15:45 ECO-O-02	Factors influencing intestinal parasite infection intensity and parasite community in juvenile spotted hyenas in the Serengeti National Park Susana Ferreira (Berlin/DE)
16:00 ECO-O-03	A glimpse of the red queen – long-term experimental co-evolution in a vertebrate-tapeworm system Marc Ritter (Plön/DE)
16:15 ECO-O-04	Cryptic species and unexpected intermediate host specificity in the acanthocephalan *Polymorphus minutus* Daniel Grabner (Essen/DE)
16:30 ECO-O-05	Anisakis infection in fish – an eco-parasitological study in different fishing grounds of the Adriatic Sea Emy Costantini (Dublin/IE)
16:45 ECO-O-06	Selection of an entomopathogenic fungus infective for ticks based on biotechnological criteria and virulence Sissy-Christin Lorenz (Bielefeld/DE)

17:00–17:20	Official closing ceremony
Lecture hall Audimax	**Oral presentation and poster awards** (see page 13)
Chair	Georg von Samson-Himmelstjerna (Berlin/DE)

09:00–16:00 Satellite workshop I • AVIAGIS

Institute Par. & Trop. Vet. Med. Room 1

” VECMAP on-tour” is Avia-GIS new initiative to organize introductory workshops hosted by institutions throughout Europe which are at the heart of the action. VECMAP on-tour workshops introduce a totally new and easy all-in-one way to map and model the distribution of disease vectors and indeed any other invasive or autochthonous species depending on the environment for its survival.
The initial aim was to PhD students to VECMAP as an easy one-stop-shop tool that supports all the steps required to map and model, at various scales, the distribution of disease vectors, to plan surveillance & control programs, and manage the entire database.The aim is now expanded to engage other players in the sector who would need such a tool to complete their projects and researches efficiently and precisely. Moreover, Decision makers can get the right visual information to take actions.
The setup of the workshop is built in an interactive way where all participants can have the opportunity to exchange knowledge, experience and inspire each other. It is a way to bridge the gap between science and decision making. The workshop includes a hands-on training on VECMAPLite software and basic GIS.

At the workshop participants will:

1. Understand what VECMAP is & its added value to their work
2. Know how to gear VECMAP to their specific needs
3. Get hands-on training on spatial modelling using VECMAPLite
4. Exchange their experiences about species mapping and the use of Spatial Decision Support Systems (SDSS) with the other participating specialists
5. Each participant will have her/his own VECMAPLite software package with training exercises.
6. Certificate of VECMAPLite training is provided at the end of the workshop
7. Access to VECMAPLite community

09:00–12:00 Satellite workshop II • Artificial tick feeding

Institute Par. & Trop. Vet. Med. Room 2

On Saturday, 24 March, from 09:00 to 12:00, a workshop on the ‘artificial feeding of ticks’ will be given at the Institute for Parasitology and Tropical Veterinary Medicine, Robert-von-Ostertag-Str. 7-13, 14163, Berlin.
During the workshop, different artificial tick feeding methods will be presented and discussed. Participants will receive hands-on experience in the main in vitro tick feeding methods, including the preparation of silicone feeding membranes.
The workshop is open for registered DGP conference participants only, with a maximum of 25 attendees. Pre-registration for this workshop is required.

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	Physics of parasitism	50
	Protozoan parasites	50
	Vectors, entomology and acarology	52
	Veterinary parasitology	52

Biochemistry

BIO-P-02 Two different thioredoxin reductases in one amoeba – the extraordinary thioredoxin-linked redox system in *Acanthamoeba castellanii*
David Leitsch (Vienna/AT)

BIO-P-04 A single-cysteine mutant and chimeras of essential *Leishmania* Erv can complement the loss of Erv1 but not of Mia40 in yeast
Sandra Specht (Kaiserslautern, Heidelberg/DE)

Cell biology and signaling

CBS-P-02 *Plasmodium falciparum* ubiquitin transferase, a novel putative quinine resistance marker
Monika Jankowska (Heidelberg/DE)

CBS-P-04 A novel Golgi-dwelling phosphatidylinositol synthase is essential for the lytic cycle of Toxoplasma gondii
Levon Ruhbach (Berlin/DE)

CBS-P-06 Development of a screening assay for regulators of protein kinase A signaling in *Trypanosoma brucei*
Qingping Wu (Martinsried/DE)

CBS-P-08 Optogenetic induction of cAMP signaling in T. gondii
Theresa Störiko (Berlin/DE)

Diagnosis

DIA-P-02 Development of an *Bartonella henselae* specific Human IgG ELISA
Markus Jost (Frankfurt am Main/DE)

DIA-P-04 Detection of Lyme Borrelia by Loop-mediated isothermal amplification (LAMP) – an in-field experience
Donato Antonio Raele (Foggia/IT)

DIA-P-06 Prevalence of *Schistosoma mansoni*, Soil-Transmitted Helminths and Intestinal Protozoa – baseline data of the Ijinga Island Schistosomiasis Elimination Pilot Study, Northwestern Tanzania
Clemens Mechler (Würzburg/DE)

DIA-P-08 A modification of the Harada-Mori-Culture for the detection of nematode larvae in fecal samples
Andreas Mueller (Würzburg/DE)

DIA-P-10 Wet mount microscopy, mini-FLOTAC and PCR for the diagnosis of Ascaris lumbricoides
Prabhanjan P. Gai (Berlin/DE)

Drugs and drug development

DDD-P-02 Studying the Chemical Composition *in vitro* Activity of *Cinnamomum zeylanicum* and *Eugenia caryophyllata* Essential Oils on *Leishmania* major
Masoud Soosaraei (Sari/IR)

DDD-P-04 Proteome Analysis of Excretory-Secretory Products of *F. hepatica* in the Presence or Absence of Triclabendazole (Anthelmintic Drug) Using Two-Dimensional Gel Electrophoresis
Sepideh Farahnak (Tehran/IR)

DDD-P-06 Structure optimization of 3,4-disubstituted Benzhydroxamates as modulators of epigenetic targets for the treatment of parasitic diseases
Tino Heimburg (Halle an der Saale/DE)

DDD-P-08 Establishment of a screening platform for testing compounds against Schistosoma mansoni in vitro
Julie Harnischfeger (Giessen/DE)

DDD-P-10 Synthesis and in vitro Testing of Dithiocarbamates as Novel Anthelmintic Inhibitors against Schistosomiasis
Georg Alexander Rennar (Marburg/DE)

Epidemiology and emerging infections

EPI-P-02 Diagnostic validation of a magnetic capture PCR for the copro-diagnosis of Echinococcus multilocularis infection in foxes using the Intestinal Scraping Technique as a reference
Pavlo Maksimov (Greifswald – Insel Riems/DE)

EPI-P-04 Free-Living Amoebae as hosts for "Giruses" and vectors of microorganisms with "Public Health" significance
Patrick Scheid (Koblenz/DE)

EPI-P-06 Cryptosporidium parvum and other intestinal parasites among diarrheal and non-diarrheal HIV positive patients in Asella teaching hospital, Ethiopia
Million Getachew Mesfun (Asella/ET)

EPI-P-08 Parasitological investigation of Eimeria spp. and haemosporidia among domestic chickens (Gallus gallus domesticus) and guinea fowls (Numida meleagris) slaughtered at two selected poultry markets in Lagos State, Nigeria
Emmanuel Idowu (Akoka/NG)

EPI-P-10 Retrospective analysis of vector-borne diseases in dogs after travelling to endemic areas (2007–2015)
Ingo Schäfer (Berlin/DE)

EPI-P-12 Screening of Mediterranean tick species by high-throughput microfluidic real time PCRs reveals a significant diversity of bacterial and parasitic pathogens with co-infections being the norm
Anastasios Saratsis (Thermi/GR)

EPI-P-14 Baylisascaris procyonis in free-ranging raccoons (Procyon lotor) in Saxony, eastern Germany
Zaida Renteria (Leipzig/DE)

Free topics

FTO-P-02 Effect of different host plants utilization on physicomorphic responses of polyphagous Helicoverpa armigera (Hübner) (Noctuidae – Lepidoptera)
Sajjad Ali (Bahawalpur/PK)

Immunology

IMM-P-02 Preclinical evaluation of transgenic Plasmodium berghei sporozoites expressing the TLR5 agonist Salmonella enterica flagellin
Katja Müller (Berlin/DE)

IMM-P-04 Immune reactions against the laboratory strain of Eimeria falcifomis are stronger than against a wild derived strain
Enas Al-khlifeh (Berlin/DE)

IMM-P-06 Cryptosporidium Infection and Correlation with CD4+ T-cell count among HIV on HAART therapy in Osogbo, Nigeria
Sulaiman Adebayo (Ogbomoso/NG)

Molecular genetics

MOL-P-02 New molecular tools for studying the biology of Leishmania mexicana
Lucie Podešvová (Ostrava/CZ)

MOL-P-04 MT10 is the most common Microsatellite Type (MT) among Trichomonas vaginalis isolates from Aydın, Turkey
Hatice Ertabaklar (Aydın/TR)

Parasite ecology

ECO-P-02 Development of a biological tick trap based on attract-and-kill strategy: Screening of attractants
Kerstin Büchel (Berlin/DE)

Parasite-host interactions

PHI-P-02 Genome-wide association study of endoparasite resistance in a population of Black and White dairy cows
Katharina May (Hanover/DE)

PHI-P-04 The importance of Plasmodium vivax VIR proteins for the cytoadhesion of infected erythrocytes
Torben Rehn (Hamburg/DE)

PHI-P-06 RNAi-based trigger gene silencing approach in Entamoeba histolytica to identify pathogenicity factors involved in amoebic liver abscess formation
Sarah Corinna Lender (Hamburg/DE)

PHI-P-08 Avian malaria on Madagascar – specialization of endemic haemosporidian parasites
Anke Dinkel (Stuttgart/DE)

PHI-P-10 The Stubborn Apicomplexan – lack of transcriptional plasticity to host immune defenses
Totta Ehret (Berlin/DE)

PHI-P-12 *Wolbachia pipientis* in natural populations of mosquito vectors of Dirofilaria from Russia
Elena Shaikevich (Moscow/RU)

PHI-P-14 "New king new law" – biting midges as a probable vector of the etiologic agent of cutaneous leishmaniasis in Ghana
Godwin Kwakye-Nuako (Cape Coast/GH)

Parasitic helminths

PHE-P-02 The Feasibility of a "Re-mapping" Protocol for Lymphatic Filariasis in Areas where Transmission is Uncertain in Ethiopia
Heven Sime Firew (Addis Ababa/ET)

PHE-P-04 Epizootiology of Fasciolosis in sheep (Ovis Aries) raising in geoclimatic setting of Poonch District of Azad Kashmir, Pakistan
Asim Shamim Shamim (Rawalaokt/PK)

PHE-P-06 *Schistosoma mansoni* infection among preschool-aged children on Ijinga Island, Northwest Tanzania – prevalence and intensity of infection
Antje Fuß (Würzburg/DE)

PHE-P-08 Iatrogenic helminth infection in a patient with systemic lupus erythematodes under hydroxychloroquine therapy – a case report
Ingrid Reiter-Owona (Bonn/DE)

Phylogeny and evolution

PAE-P-04 Ecology of Corynosoma infection in Sea Otters and Seals as an example of parasite diversity and evolutionary divergence.
Kyle Shanebeck (Büsum/DE)

PAE-P-06 Survey of Haemosporidian Parasites in Bats in Ngounié Province, Gabon
Sascha P. Klose (Berlin/DE)

Physics of parasitism

POP-P-02 Structural basis for the protective function of the dynamic variant surface glycoprotein coat of *African trypanosomes*
Nicola Jones (Würzburg/DE)

Protozoan parasites

PRO-P-02 *Babesia* spp. in wild cervids species
Irma Ražanskė (Kaunas/LT)

PRO-P-04 Declining trend of malaria and high efficacy of Artemether-Lumefantrine(Coartem®) against *P. falciparum* in Ziway Dugda district, Ethiopia
Million Getachew Mesfun (Asella/ET)

PRO-P-06 Mutual influences of the apicomplexan parasites *Toxoplasma gondii* and *Eimeria tenella* in poultry macrophages
Runhui Zhang (Leipzig/DE)

PRO-P-08 Sarcocystis nesbitti in Australia? New discovery raises question about lifecycle
Marion Wassermann (Stuttgart/DE)

PRO-P-10 Phylogeography of dsRNA viruses of *Leptomonas pyrrhocoris* (*Trypanosomatidae*, *Kinetoplastea*)
Diego Henrique Fagundes Macedo (Ostrava/CZ)

PRO-P-12 Profiling of membrane transport protein expression in the *Plasmodium berghei* life cycle exemplified by CRT and ATP4
Francois Korbmacher (Berlin/DE)

PRO-P-14 Generation and characterization of selected virulence factor knockout strains of C. parvum using CRISPR/CAS method
Maxi Berberich (Leipzig/DE)

PRO-P-16 In vitro investigation of three oocyst specific proteins and their role for survival of Toxoplasma gondii oocysts in the environment
Benedikt Fabian (Berlin/DE)

PRO-P-18 Comparison of the LightMix® Modular Assay Gastro Parasites with routine laboratory diagnosis for the detection of parasites in stool specimens from primary health care patients in Berlin, Germany
Johannes Friesen (Berlin/DE)

PRO-P-20 Deciphering the molecular machinery of the antigenic variation regulator DOT1B
Nicole Eisenhuth (Würzburg/DE)

PRO-P-22 Identification of novel components of the histone methyltransferase DOT1A protein complex in Trypanosoma brucei
Tim Vellmer (Würzburg/DE)

PRO-P-24 The putative 2,4-dienoyl-CoA reductase of Leishmania represents a novel virulence factor
Geo Semini (Berlin/DE)

PRO-P-26 Estimating Apicomplexan parasite exposure in Icelandic arctic foxes (Vulpes lagopus)
Gábor Árpád Czirják (Berlin/DE)

PRO-P-28 *In vivo* effect of Coenzyme Q10 on the generation and regulation of pathologic immune responses in Plasmodium infection
James Nyabuga Nyariki (Nairobi/KE)

PRO-P-30 Investigations in Sarcosporidia in native rodents and treeshrews from Borneo
Paula Ortega Pérez (Berlin/DE)

PRO-P-32 Bumped Kinase inhibitor 1294 and its effect on protein expression and localization in *N. caninum*
Pablo Winzer (Bern/CH)

Vectors, entomology and acarology

VEA-P-04 Avian malaria on Madagascar – bird hosts and putative vector mosquitoes of different Plasmodium lineages
Anke Dinkel (Stuttgart/DE)

VEA-P-06 Study on Ticks infection (Argasidae and ixodidae) to Anaplasmosis eastnorth Iran
Zakkyeh Telmadarraiy (Tehran/IR)

VEA-P-08 Origins of recently emerged foci of the tick Dermacentor reticulatus in Poland inferred from molecular markers
Anna Bajer (Warsaw/PL)

VEA-P-10 Flourishing in germs – Deciphering the role of bacteria in development of the malaria vector Anopheles coluzzii
Caroline Kiuru (Berlin/DE)

VEA-P-12 Prospection of Simulium flies in Tunisia
Rahel Sarah Schnell (Tübingen/DE)

Veterinary parasitology

VPA-P-02 Detection of Neospora caninum-specific antibodies in breeding bitches
Rodolfo Villagra-Blanco (Giessen/DE)

VPA-P-04 Microsatellite analysis by FLA provides markers for strain discrimination of geographically close isolates of Cystoisospora suis
Anja Joachim (Vienna/AT)

VPA-P-06 Analysis of molecular mechanisms involved in Besnoitia besnoiti-mediated NETosis
Ershun Zhou (Giessen/DE)

VPA-P-08 Fox in trouble. Tick-borne microparasites affecting red fox (Vulpes vulpes) in Poland
Ewa Julia Mierzejewska (Warszawa/PL)

VPA-P-10 Molecular infection prevalence of ruminants" Tunisian meat by three protozoa – Toxoplasma gondii, Neospora caninum and Sarcocystic spp.
Mohamed Gharbi (Sidi Thabet/TN)

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Biochemistry

BIO-P-01 Identification of anti-microbials and their mode of action against persisting stages of Toxoplasma gondii
Jens Pikkemaat (Berlin/DE)

BIO-P-03 The cytosolic glyoxalases of Plasmodium falciparum are dispensable during asexual blood-stage development
Cletus Wezena, Romy Alisch (Heidelberg/DE)
Linda Liedgens (Kaiserslautern, Heidelberg/DE)

Cell biology and signaling

CBS-P-01 Comparative characterization of the putative serpentine receptors SR10 and SR12 in the malaria parasite *Plasmodium falciparum*
Monika Saini (Aachen/DE)

CBS-P-03 Characterization of adenylate cyclases in *Toxoplasma gondii*
Matthias Noll (Berlin/DE)

CBS-P-05 BioID-based proximity screening for interaction partners of *Trypanosoma brucei* protein kinase A
Kristina Malenica (Martinsried/DE)

CBS-P-07 Optogenetic regulation of cGMP signaling in *Toxoplasma gondii*
Claudia Ufermann, Elena Pies (Berlin/DE)

Diagnosis

DIA-P-01 Molecular Detection of *Theileria annulata* in Cattle Suffering from Respiratory Affections
Laila Ahmed (Assiut/EG)

DIA-P-03 A novel Loop-mediated isothermal amplification (LAMP) assay for the detection of Rickettsiae belongs the spotted fever group and typhus group
Donato Antonio Raele (Foggia/IT)

DIA-P-05 A quantitative real-time Polymerase Chain Reaction for the specific detection of *Hammondia hammondi* and its differentiation from *Toxoplasma gondii*
Gereon Schares (Greifswald – Insel Riems/DE)

DIA-P-07 A prospective evaluation of an automated detection of malaria parasites using the CellsCheck ™ compared to expert microscopy
Karin Ludwig (Tübingen/DE)

DIA-P-09 Performance of a malaria rapid diagnostic test, CareStart™ Malaria HRP2/pLDH (pf), and diversity of P. falciparum histidine-rich proteins 2 and 3 in Busia County, Western Kenya
David Nderu (Tübingen/DE; Kerugoya/KE)

Drug resistance

DRE-P-01 Effect of albendazole and mebendazole on drug-metabolizing enzymes in *Hymenolepis diminuta*
Ivan Vokral (Hradec Kralove/CZ)

Drugs and drug development

DDD-P-01 Medicinal plants with promising antileishmanial activity in Iran: asystematic review and meta-analysis
Masoud Soosaraei (Sari/IR)

DDD-P-03 Evaluating the Effect of Protein Kinase Inhibitor (imatinib) in comparison to Praziquantel on *Schistosoma mansoni* Infection
Maisa Kamel, Shimaa Helmy, Enas Rizk, Amira Raafat (Cairo/EG)

DDD-P-05 Structure-Based Design and Synthesis of Dual Targeting Inhibitors for the Treatment of Parasitic Infections
Ehab Ghazy (Halle an der Saale/DE)

DDD-P-07 Control of intracellular *Leishmania* major infection by treatment with synthetic analogs deduced from an *Entamoeba histolytica* glycolipid
Siew Ling Choy (Hamburg/DE)

DDD-P-09 Antileishmanial activity and in silico mechanism of action of fucosterol isolated from the brown seaweed *Sargassum vulgare* C. Agardh
Lauve Rachel Tchokouaha Yamthe (Accra/GH; Yaounde/CM)

DDD-P-11 Synthesis and biological evaluation of biarylalkylcarboxylic acid derivatives
Alejandra M. Peter Ventura (Marburg/DE)

Epidemiology and emerging infections

EPI-P-01 Bartonella spp. in Domestic and Wild Animals and their Ticks in Hesse, Germany – Serology, PCR and Microbiome analysis
Yvonne Regier (Frankfurt am Main/DE)

EPI-P-05 Molecular Epidemiology and Future Projection of Cutaneous Leishmaniasis in Libya Untill 2060
Ahmad Amro (Berlin/DE)

EPI-P-07 Epidemiology of Crimean-Congo Hemorrhagic Fever in Livestock animals of Balochistan, Pakistan – a risk indicator for the human population
Khushal Khan Kasi (Greifswald – Insel Riems/DE)

EPI-P-09 Comparative study of toxoplasmosis amongst healthy volunteers and Schizophrenics attending two Health Facilities in Port Harcourt, Rivers State, Nigeria
Gloria Ngozika Wokem (Port Harcourt/NG)

EPI-P-11 Characterization of Trypanosoma sp. circulating in Nigeria and Southern Chad
Judith S. Weber, Mahamat A. M. Ibrahim (Bremen/DE)

EPI-P-13 Apicomplexan and helminth parasite infections of free-ranging cheetahs (Acinonyx jubatus) on Namibian farmland
Gábor Árpád Czirják (Berlin/DE)

EPI-P-15 Prevalence of kdr-genotype of German headlice and relevance for pediculosis treatment
Anton Aebischer (Berlin/DE)

Free topics

FTO-P-01 Parasitological field work in an era of hyper regulation and the role of Museums collections
Jean Mariaux (Geneva/CH)

Immunology

IMM-P-01 Effect of the antimalarial drug pyrimethamine on the resolution of experimental cerebral malaria.
Rituparna Bhattacharjee (Magdeburg/DE)

IMM-P-03 The importance of Mansonella perstans infection – an immuno-epidemiological study in Ghana
Norman Nausch (Düsseldorf/DE)

IMM-P-05 Host age and background affect the phenotype of GATA-3 expressing CD4+ T-cell in nematode infection
Nicole Affinass (Berlin/DE)

IMM-P-07 Acute toxoplasmosis and the hyporesponsiveness of splenocytes
Christoph-Martin Ufermann (Düsseldorf/DE)

Molecular genetics

MOL-P-01 RNAi mediated knockdown of targeted genes in Strongyloides ratti
Alex Dulovic (Tübingen/DE)

MOL-P-03 Development of CRISPR/Cas9-based reverse genetics for *Leishmania braziliensis* and application to the study of differentially expressed target genes
Vanessa Adaui (Hamburg/DE)

MOL-P-05 Expression and Purification of Recombinant *Toxoplasma gondii* Vaccine Candidates (SAG1 and Cyc18) in Leishmania tarentolae
Dalia Ahmed (Glasgow/GB, Baghdad/IQ)

Parasite ecology

ECO-P-01 Malaria and intestinal parasites in pregnant woman at Abobo district (Abidjan, Côte d'Ivoire)
Gaoussou Coulibaly (Abidjan/CI)

Parasite-host interactions

PHI-P-01 Eimeria bovis macromeront formation in endothelial host cells – role of sterol uptake and transport
Liliana M.R. Silva (Giessen/DE)

PHI-P-03 Identification of Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) molecules involved in the interaction with various human endothelial receptors
Lisa K. Roth (Hamburg/DE)

PHI-P-05 Development of tsetse fly-transmitted African trypanosomes in human skin tissue models
Christian Reuter (Würzburg/DE)

PHI-P-07 Carrion crows (Corvus corone) of southwest Germany – important hosts for haemosporidian parasites
Anke Dinkel (Stuttgart/DE)

PHI-P-09 Drug-induced clearance of helminth infection restores efficacy of anti-Influenza vaccination
Nadine Stetter (Hamburg/DE)

PHI-P-11 Characterizing protein function at the parasite – host interface during blood stage infections of *Plasmodium berghei*
Julie Anne Gabelich (Berlin/DE)

PHI-P-13 Deciphering P. berghei EXP-1 function during pre-erythrocytic and erythrocytic development at the host-parasite interface
Kamil Wolanin (Heidelberg/DE)

PHI-P-15 Cross-species correlation of host and parasite gene expression as a tool to identify protein interactions between Plasmodium and hosts
Parnika Mukherjee (Berlin/DE)

Parasitic helminths

PHE-P-01 Experimental Study on Life Cycle of *Hypoderaeum conoideum* (Block, 1872) Diez, 1909 (*Trematoda – Echinostomatidae*) Parasite from the North of Iran
Ali Farahnak (Tehran/IR)

PHE-P-03 Strongyloides stercoralis in humans and dogs – a study in northern Cambodia
Siyu Zhou (Tübingen/DE)

PHE-P-05 *Schistosoma mansoni* histone deacetylase 8 (SmHDAC8) interacts with the Rho GTPase SmRho1
Lucile Pagliazzo (Lille/FR)

PHE-P-07 Identification of helminths in house mice from the European Hybrid Zone – combining classic taxonomy and a molecular approach
Jenny Jost (Berlin/DE)

Phylogeny and evolution

PAE-P-01 Molecular Identification and Characterization of *Theileria* spp responsible for Ovine Theileriosis in Egyptian Oases
Amira AL-Hosary (Assiut/EG)

PAE-P-03 Diversity of *Eimeria* spp. in different mouse genotypes across the European Hybrid Zone
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ABSTRACTS

ABSTRACTS

Plenary abstracts

PL-O-01

Symbiosis and parasitism in filarial nematodes and their *Wolbachia endobacteria*

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The sequencing of the genomes of parasitic organisms has several goals including the definition of biochemical and physiological systems to support drug development activities, identifying the full repertoire of molecules interacting with immune systems to support immunological nterventions, and as a substrate for models of population structure and dynamics to support epidemiological analysis. Parasite genomes also contain a record of the evolutionary history of each species, revealing its closest neighbours, and its deep relationships with others, including non-parasites. While genomic analyses of the ultimate origins of parasitism are often obscured by subsequent millenia of adaptation to the lifestyle, more recent transitions in parasitic lifecycles are more amenable to analysis. One such transition is the acquisition of an alphaproteobacterial endosymbiont, *Wolbachia,* by filarial nematodes. *Wolbachia* are common alphaproteobacterial endosymbionts of terrestrial arthropods and nematodes. In most arthropods, *Wolbachia* behave as reproductive parasites, while in nematodes they may be essential, mutualist symbionts. Filaria (Onchocercinae) are nested within a monophyletic clade of parasites (Spirurina, Clade III), and their closest relatives do not carry *Wolbachia* infection. Thus this acquisition is relatively recent. We have used genomics to answer several outstanding questions about this symbiosis, including how often filaria have acquired *Wolbachia* and the essentiality of the relationship between host and symbiont.
Filarial nematodes harbour a range of different strains of *Wolbachia* that are classified into different supergroups. Using nematode and symbiont genomic data we have tracked the evolution of the association between *Wolbachia* and their nematode hosts. *Wolbachia* DNA is frequently laterally transferred into the host genome, and these insertions reveal the palaeobiology of extinct symbionts and the dynamics of symbiont acquisition and loss. Our analyses reveal at least two invasions of filarial nematodes by Wolbachia in filarial nematodes, and show that the supergroup D symbionts of *Brugia* and related species are latecomers to this important symbiosis. Several species have lost their symbionts, as evidenced by the presence of *Wolbachia* DNA in their nuclear genomes in the absence of a live *Wolbachia* infection.

PL-O-02

MicroRNAs in nematode drug resistance and host-parasite interactions

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microRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression at the post-transcriptional level. They are expressed in a diverse range of organisms from viruses to humans. We are examining miRNAs in parasitic nematodes to investigate their regulatory roles in parasite development, immune modulation and drug resistance. Focussing on the ovine blood-feeding

gastrointestinal nematode *Haemonchus contortus*, we identified 192 miRNAs and examined their developmental expression using microarrays. A number of miRNAs are expressed in a developmental- and tissue-specific manner. In addition, they are present in excretory-secretory (ES) products and extracellular vesicles (EVs) released by parasitic stages, suggesting a potential role in modulating host immunity. We also detected significant upregulation of one miRNA, hco-mir-9551, in two genetically distinct strains of *H. contortus* resistant to ivermectin. This miRNA may be responsible, directly or indirectly, for changes in gene expression leading to resistance. miRNAs are important in understanding parasite development, host-parasite interactions and drug resistance and our findings highlight the potential of miRNAs and the pathways they regulate as novel targets for parasite control.

PL-O-03
Regulation of adaptive immunity during *Plasmodium* infection

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Adaptive immunity is essential for controlling infection during blood stage propagation of the parasite *Plasmodium*. These immune responses have to be tightly balanced to guarantee parasite clearance without induction of severe immunopathologies, emphasizing the need for immunoregulatory mechanisms. $CD4^+Foxp3^+$ regulatory T cells (Tregs) have been shown to expand in *Plasmodium*-infected patients and mice. These cells play a crucial role in regulating immune responses during *Plasmodium yoelii* infection, as Treg depletion resulted in elevated T cell activation accompanied by enhanced pathogen clearance. In addition to $Foxp3^+$ Tregs, we identified another immunosuppressive IL-10-secreting $CD4^+$ T cell population (Tr1 cells) during parasite infection. Further analysis provided evidence that the induction of Tr1 cells is not a direct effect caused by the parasite, but rather an indirect consequence due to T cell activation by a subset of dendritic cells (DCs) with elevated IL-10 expression upon infection. In general, DCs act as antigen-presenting cells and are known to be important for orchestrating effector T cell responses. However, during *Plasmodium* infection, DCs play a crucial role in T cell activation only during the initial phase, while being dispensable at later time points

PL-O-04
The complexity and the simplicity of host-*Plasmodium* interactions

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Despite renewed eradication efforts from the international community, malaria still exerts an enormous disease burden, with nearly half the planet"s population at risk of infection. Within the human host, the disease-causing Plasmodium parasites pass through two distinct lifecycle stages, each in a different cellular environment. During the liver stage, a single Plasmodium sporozoite will invade a hepatocyte, and while sheltered there, supposedly undetected by the host, gives rise to thousands of new parasites, which will go on to initiate the subsequent blood stage of infection. While only 10-20 new parasites will be generated inside an erythrocyte, consecutive cycles of cell lysis and reinfection

causing a potent host response, as well as the symptoms of malaria. The host contribution to infection outcome, on both the cellular and organismal levels has recently moved to center stage. We have identified hepatocyte molecules that modulate the success of liver stage infection, and showed that distinct host factors, not just the parasite itself, drive the onset and severity of diverse malaria syndromes. Our ongoing work indicates that the web of host-Plasmodium interactions is densely woven, with liver stage-mediated innate immune system activation, host nutritional status, and an antagonistic relationship between the two parasite stages themselves all working to modulate the balance between parasite replication and human health.

PL-O-06
Theileria parva live and subunit vaccines and genomics: an overview

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Theileria parva is a tick-transmitted apicomplexan protozoan parasite that immortalizes bovine lymphocytes, resulting in a rapidly lethal disease, particularly in susceptible taurine cattle. An infection and treatment procedure (ITM) involving inoculation of a lethal dose of live sporozoites and a long acting formulation of oxytetracycline was developed in the mid1970s, but deployment was limited until recently. One mechanism of protection induced by ITM is class I-MHC restricted cytotoxic T lymphocytes. Research to produce an anti-schizont recombinant vaccine targeting this response has resulted in scientific milestones, including candidate CTL target antigen identification, but due to the lack of an effective antigen delivery system no effective experimental vaccine has been developed. An anti-sporozoite-based vaccine, p67, consistently results in 70% protection in the laboratory, but has so far failed to protect significantly against field tick challenge. In the interim, despite production constraints and high delivery costs, several million doses of ITM have now been delivered, primarily to pastoralists in Tanzania and Kenya. Complete genome sequencing reveals high levels of diversity, recombination and gene conversion in T. parva from cattle, which are higher than between isolates of Plasmodium falciparum. Surprisingly the p67 gene is totally conserved in cattle transmissible parasites. Genotyping reveals transfer of the ITM immunizing genotypes into naïve tick and cattle populations in the field, although the very extensive diversity of the parasite makes it unlikely that this will affect ITM sustainability. Currently almost nothing is known about mechanisms of immunity induced by delivery of sporozoites by the tick, or why cape buffalo are typically multiply infected, but do not exhibit clinical symptoms.

PL-O-07
Parasite diversity and competitor-predator-prey relationships: recolonising grey wolves in central Europe as a natural experiment

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The recent recolonisation of Central Europe by the grey wolf (*Canis lupus*) provides a rare, quasi-experimental opportunity to study the dynamics of parasite transmission and their link to predator-prey interactions for cases when a definitive host returns after a phase of local extinction. We investigated parasite richness and diversity in grey wolves, their prey (red deer *Cervus elaphus*, roe deer *Capreolus capreolus* and wild boar *Sus scrofa*) and domestic dogs used by hunters with classical methods and high throughput sequencing techniques. We asked which role do prey and predator populations play in the re-establishment of endoparasite life cycles, which intrinsic and extrinsic factors control individual endoparasite diversity in an expanding host population, whether wolves increased the prevalence of parasites for which ungulate prey acts as intermediate host, whether some parasite species are particularly well adapted to wolves, what the mechanism of such adaptations might be and whether domestic dogs used for hunting are at an increased risk of parasite transmission in wolf areas. Our findings indicate that immigrated wolves increase parasite diversity in German packs, and prevalence of wolf-associated parasites had declined during wolf absence and has now risen during recolonisation. Predator–prey interactions influenced parasite prevalence if both predator and prey are part of the parasite life cycle and age–dependent immune maturation contributes to the control of protozoan infection in wolves. General parasite burden in dogs used for hunting was not increased by wolves, although the "wolf specialist" parasite *Sarcocystis grueneri* was more prevalent in dogs used for hunting in areas where wolves are present. We conclude that mesopredators did not replace the apex predator as definitive host during its absence and that temporal dynamics in predator-prey-parasite relationships are likely to be underestimated

Orals

Biochemistry

BIO-O-01
Comparative Nematode Glycomics

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Question: Glycans cover the surfaces of all cells and are frequently components of secreted proteins, whether of host or parasite origin. Of the various types of glycan that exist in eukaryotes, we focus on those covalently linked to asparagine residues of proteins (N-glycans) and have recently examined the N-glycomes of *Oesophagostomum dentatum*, *Haemonchus contortus* and *Trichuris suis* in order to prove which types of glycan epitope are present in these species.

Methods: We have adopted an analytical glycomic workflow with glycan release, labelling and HPLC fractionation followed by MALDI-TOF mass spectrometry in combination with enzymatic or chemical treatments and MS/MS fragmentation.
Results: Each of the glycomes studied had unique as well as conserved features. Amongst the common features is the presence of phosphorylcholine (PC), but *O. dentatum* has extended PC-decorated GlcNAc-based chains, while *T. suis* has PC in the context of fucosylated and non-fucosylated forms of LacdiNAc. Both *H. contortus* and *O. dentatum* have trifucosylated N-glycan cores in part modified with galactose, but *T. suis* glycans have maximally two fucose residues.
Conclusion: Parasite glycomes are, thereby, also quite different as compared to their mammalian hosts and the exact knowledge of glycan structures has repercussions for production of recombinant glycoprotein vaccines and for an understanding of the interaction of parasites with host immune systems.
References: Biochim Biophys Acta 1861:418-430, Anal Bioanal Chem 408:461-71, Glycobiology 25:585-90

BIO-O-02
Comparative Trichomonad Glycomics

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Question: Trichomonad species are widespread unicellular flagellated parasites of vertebrates which interact with their hosts through carbohydrate-lectin interactions. In the past, some data has been accumulated regarding their lipophosphoglycans, a major glycoconjugate on their cell surfaces; on the other hand, other than biosynthetic aspects, few details about their N-linked oligosaccharides are known. In this study, we compared different strains of *Trichomonas vaginalis*, a parasite of the human reproductive tract, as well as a strain of *Tritrichomonas foetus*.
Methods: We employed off-line HPLC-MALDI-TOF MS in order to compare the structures of the released N-glycans.
Results: A major structure is a truncated oligomannose form (Man5GlcNAc2), compatible with a previous bioinformatic examination of the glycogenomic potential of *T. vaginalis*. In addition, dependent on the strain, N-glycans modified by pentose residues, phosphate or phosphoethanolamine and terminal N-acetyllactosamine (Galβ1,4GlcNAc) units were found. In *T. foetus*, the range of structures overlapped with that in *T. vaginalis*, but was not identical.
Conclusion: The modification of N-glycans by N-acetyllactosamine in at least some strains is shared with the lipophosphoglycan and may represent a further interaction partner for host galectins, thereby playing a role in binding of the parasite to host epithelia. On the other hand, the variation in glycosylation between strains may be the result of genetic diversity within the species.
Reference: Glycobiology 22:300-13

BIO-O-03
Understanding the biological role of parasitic N-glycans using microarrays

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Question: Glycosylation of proteins is one of the most important post-translational modifications and important for protein stability, secretion and localization. In the case of parasitic nematodes, N-glycans frequently terminated with the zwitterion phosphorycholine can be identified. This specific modification seems to be biologically relevant as it is required for immunomodulatory effects; however, specific methods to test interactions with natural glycans need to be developed.
Methods: To better define the biological function of these parasitic sugar modifications, N-glycans from the dog parasite *Dirofilaria immits* were prepared, derivatized with a bivalent fluorescent tag, analyzed by mass spectrometry and HPLC methods and finally used to generate a defined carbohydrate microarray. The array was probed with sera from non-infected as well infected dogs, various glycan- binding proteins, pentraxins and antibodies.
Results: Using this approach, structures containing non-mammalian elements such as phosphorylcholine and multiple fucosylation on the antennae could be identified. These elements could be immunogenic to the dog host; however, on the glycan microarray only a weak binding signal of carbohydrate-binding IgM could be determined. Also, mannose-binding lectin failed to recognize the natural parasitic sugar molecules. Only after the chemically removal of the parasite-type modifications was binding to the glycans detected. In contrast, the pentraxin CRP was found to be present in the dog sera and could bind natural glycans in combination with the complement factor C1q.
Conclusion: Taken together, the results show that the N-glycans of *D. immitis* contain a number of features, which may mediate immunomodulation in the host or confer the ability to avoid immune surveillance.

BIO-O-04
Structural analyses of nematode N-glycan post-translational modifications elucidate conserved functional epitopes and biosynthetic pathways

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Question: Nematodes constitute a large number of different species on earth and some of them are parasites to mammals causing diseases. Glycoproteins, synthesised by nematodes, are decorated with a different set of glycan structures in comparison to the ones produced by mammals. Nematode glycans could be recognised by the immune system during the course of parasite-host interactions, and some of the glycans even have immunomodulatory functions. To better understand the N-glycosylation machinery in nematodes, we use the free-living *Caenorhabditis elegans* as a model.

Methods: *C. elegans* mutants were generated to investigate the function of genes which are involved in N-glycan biosynthesis. An off-line HPLC-MALDI-TOF-MS strategy in combination with exo-glycosidases and chemical treatments was employed to profile and sequence N-glycan structures from both parasitic species and free-living species in a great detail. Furthermore, a number of fucosyltransferases (FUTs), hexosaminidases and *N*-acetylglucosyltransferases were cloned and recombinantly expressed so that their functions were studied by in vitro glycan remodelling.
Results: A range of conserved functional epitopes on N-glycans were characterised in *C. elegans* wild-type and mutant strains and were also identified in parasitic species, such as *Oesophagostomum dentatum* and *Haemonchus contortus*; this includes different types of Gal-Fuc modifications of the chitobiose core and phosphorylcholine substitution on the N-acetylglucosamine residues of the antennae. The functions of three core-modifying FUTs were elucidated by *in vitro* and glycomic analyses.
Conclusion: The knowledge on these nematode glycoepitopes could facilitate the design of novel diagnostic strategies upon parasitic infections and encourage engineering glycovaccines in the future.
References: J Proteome Res 14:5291-305; Mol Cell Proteomics 14:2111-25; J Biol Chem 288:21015-28; J Biol Chem 287:28276-90

BIO-O-05
The metabolic role of amylopectin turnover in *Toxoplasma gondii*

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Toxoplasma gondii is an intracellular parasite that chronically infects most warm-blooded animals. It synthesizes a plant-type storage polysaccharide called amylopectin that accumulates to very high levels in the persisting bradyzoite form of the parasite. While the importance of amylopectin for chronic infections has been established it is currently not understood amylopectin is needed for efficient cyst development. Polysaccharides are often considered as static sugar and energy storage but also have been shown to fulfil regulatory metabolic functions.
Here, we used reverse genetics and stable isotope-resolved gas and liquid chromatography-coupled mass-spectrometry to investigate the role of amylopectin metabolism in *T. gondii*. We found that tachyzoites use exogenous glucose to quickly turn over a small amylopectin pool during their in vitro growth. *T. gondii* expresses two phosphoglucomutases (TgPGM1 and TgPGM2) in its cytosol that are implicated in the pathway. Genetic depletion parasite phosphoglucomutase activity in a *Δpgm1/2* strain incurs only a mild growth defect in tachyzoites. While, *Δpgm1/2* mutants are able to synthesize amylopectin at a reduced rate they appear defective in amylopectin utilization and accumulate high levels of UDP-sugars. Untargeted LCMS-based metabolomics shows that disturbed amylopectin turnover leads to global changes in parasite metabolism and affects glycolytic flux, the pentose phosphate pathway and fatty acid synthesis. Together, our results suggest that amylopectin turnover influences several major glucose utilization pathways whose efficient regulation might be required during cyst development.

BIO-O-06

***Toxoplasma gondii* harbors a pathway to synthesize amino acids otherwise essential for its mammalian host cells**

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The amino acids of the aspartate family, namely lysine, methionine, isoleucine and threonine are essential to the mammalian hosts of *T. gondii*. However, plants, fungi, prokaryotes and selected eukaryotic microorganisms can produce these amino acids. We identified four out of five enzymes in the genome of *T. gondii* involved in threonine synthesis, namely aspartate kinase (AK), aspartate semi-aldehyde dehydrogenase (ASDH), homoserine kinase (HK) and threonine synthase (TS). Ectopic expression of epitope-tagged proteins revealed a cytosolic localization of the entire pathway. ASDH and HK can functionally complement the growth of the corresponding yeast mutants auxotrophic for threonine. Likewise, a transgenic parasite strain overexpressing TS can survive the lack of threonine in cultures, whereas the parental strain ceases to grow. The TS gene could be deleted in the parasite, indicating that *T. gondii* is also able to salvage threonine from host cells. Unlike TS, the first enzyme of the pathway (AK) seems essential, because it is refractory to gene deletion. Besides generating additional mutants, the current work involves testing the physiological relevance of this pathway under specific nutritional conditions, potentially encountered in diverse host-cell types.

Biochemistry and molecular genetics

BMG-O-01

A synthetic promoter for multi-stage expression reveals complementary functions of *Plasmodium* adhesins

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Gene expression of malaria parasites is mediated by the apicomplexan apetala2 (ApiAP2) transcription factor family. Different ApiAP2s control gene expression at distinct stages in the complex life cycle of the parasite ensuring timely expression of stage-specific genes. ApiAP2s recognize short cis-regulatory elements, which are enriched in the upstream/promoter region of their target genes. This should in principle allow the generation of synthetic promoters that drive gene expression at desired stages of the *Plasmodium* life cycle. Here we present a proof of concept by combining the cis-regulatory elements of two genes expressed successively within the mosquito part of the life cycle. Our tailored synthetic promoter named Spooki activates gene expression in early and late mosquito stages as shown by the expression of a fluorescent reporter. We used Spooki to address the specific functionality of two related stage-specific adhesins that are exclusively expressed either during the early or late mosquito stage. By modifying the expression profile of both adhesins in absence of their counterpart we could show that these proteins partially complement parasite gliding which suggests a common mode of action.

BMG-O-02

Interdomain GFP-tagging of the Plasmodium circumsporozoite protein visualizes sporozoite formation and reveals C-terminal processing

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Abstract
The circumsporozoite protein (CSP) is the major surface protein of Plasmodium sporozoites and essential for sporozoite formation within the oocyst (Menard et al, 1997). The details of oocyst development and sporozoite formation are difficult to investigate due to the thick oocyst wall and have mostly been studied by electron microscopy.
Here we employed interdomain tagging of CSP and CSP truncations with GFP to investigate its role during sporozoite formation. These mutants were investigated using life cell microscopy of complete midguts and electron microscopy of midgut sections. Plasma membrane invagination, resulting in sporoblast formation, before the formation of subpellicular microtubules correlated with successful sporozoite formation. Comparative western blotting using a GFP specific antibody showed that CSP undergoes a previously unrecognized proteolytic processing at the C-terminus. This was also observed in wild type oocysts using a C-terminal antibody but not in sporozoites.
Additionally we investigated membrane dynamics of gliding sporozoites using TIRF-microscopy, suggesting a previously unobserved mode of trail formation.

BMG-O-03

Gain and loss of small RNA classes in Strongyloididae

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Introduction: Small RNAs (sRNAs) play important roles in the control of transposable elements and gene expression. Nematodes differ in several ways from other taxa in their sRNAs. In particular, they have a class of 5'-triphosphorylated secondary siRNAs, which are formed by RNA dependent RNA polymerases without 5' end processing. Recent comparative studies revealed that there is also considerable variability within the nematodes. However, no Strongyloididae species or close relative was included.
Objective: Characterize the sRNA repertoire of Strongyloididae
Materials and Methods: We sequenced the sRNAs of two developmental stages of three Strongyloididae (*Strongyloides ratti*, *S. papillosus* and *Parastrongyloides trichosuri*) and compared them with the model nematodes *Caenorhabditis elegans* and *Pristionchus pacificus*. For each sample, we prepared libraries with and without treatment of the RNA with Tobacco Acid Pyrophosphatase (TAP) thereby including or excluding 5'-triphosphorylated RNAs.
Results: The 5'-monophoshate-only samples were dominated by microRNAs in Strongyloididae, as in the model nematodes. The typical piRNA (21U RNA) signal was absent in Strongyloididae and their

genomes encode no proteins of the Piwi family, which interact with piRNAs. TAP treatment led to a strong enrichment of the signal corresponding to the known 22G secondary siRNAs in *C. elegans* and *P. pacificus*. In Strongyloididae, instead of 22G RNAs there exists a novel class of around 27 nucleotide long RNAs starting with 5'G or A (27GA RNAs). A large fraction of the 27GA RNAs have the potential to target transposable elements but also other genes.
Conclusions: Strongyloididae have conserved and taxon-specific micro RNAs. The 27GA RNAs are a novel class of presumably 5'-triphosphorylated RNAs, which are probably the equivalent to the *C. elegans* 22G secondary siRNAs. Like other nematodes outside of the major clade V, which contains *C. elegans* and hookworms, Strongyloididae have no piRNAs.

BMG-O-04
The regulatory subunit of a PKA like kinase in *Trypanosoma brucei*: structural studies for novel tools

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Protein kinase A (PKA) plays an essential role in the transduction of cellular signal thereby controlling several cell functions, including regulation of glycogen, sugar and lipid metabolism. This role is conserved in a range of organisms from yeast to human. In all PKAs the kinase catalytic subunit (PKAC) is maintained inactive upon interaction with a regulatory subunit (PKAR), forming a holoenzyme complex which dissociates upon binding of cyclic AMP to the R subunit. In our laboratory it was observed that *Trypanosoma brucei* PKA is not activated by cyclic AMP. Following this observation, a set of compounds were tested by *in vivo* and *in vitro* assays and activators were identified. The best hit found is very potent in terms of binding and activation (Kd and EC50 in the low nano molar range). In order to gain insight into the architecture of the binding pockets upon interaction with different ligands, we have developed an efficient method for ligand exchange and co-crystallization that can yield high resolution models in a short period of time. We have so far 4 crystal structures of PKARs (2 from *T. brucei* PKAR and 2 from *T. cruzi* PKAR) with resolutions up to 1Å. Our aim now is to perform a structure-based virtual screening in order to find not only activators that can be used as tools for investigating the role of PKA activation in the cell, but convert them to inhibitors with potential for drug development.

1) Kim C, Cheng CY, Saldanha SA, Taylor SS., PKA-I holoenzyme structure reveals a mechanism for cAMP-dependent activation. Cell. 2007 Sep 21;130(6):1032-43

BMG-O-05
The structure of serum-resistance-associated protein and its implications for Human African Trypanosomiasis

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Only two trypanosome subspecies are able to cause Human African Trypanosomiasis. To establish an infection in human blood, they must overcome the innate immune system by resisting the toxic effects of the trypanolytic factors TLF1 and TLF2. These lipoprotein complexes contain an active component, apolipoprotein L1, ApoL1, a pore-forming component that causes trypanosome cell death by a yet not well-characterised mechanism. One of the two human infective subspecies, *Trypanosoma brucei rhodesiense,* differs from non-infective trypanosomes solely by presence of the serum-resistance-associated protein, SRA, which binds directly to ApoL1 and blocks its pore-forming capacity. Since this interaction is the single critical event that renders *T. b. rhodesiense* human infective, detailed structural information that allows identification of binding determinants is crucial to understand immune escape of the parasite. Here we present the crystal structure of SRA and reveal the adaptations that occurred as it diverged from other trypanosome surface molecules to neutralise ApoL1. In order to map the ApoL1 binding site on SRA we carried out hydrogen-deuterium exchange mass spectrometry, HDX-MS, using recombinantly expressed proteins. To confirm and to further delineate the region of SRA identified as a hot-spot for ApoL1 binding, binding affinities of wild-type and mutant SRAs to ApoL1 were determined using microscale thermophoresis. Our structure-guided studies revealed that polar residues form the core of the interface with ApoL1, rendering the interaction electrostatic in nature. These results give molecular insight into the SRA-ApoL1 interaction, providing a significant step forward in understanding the structural basis for the one critical interaction that allows *T. b. rhodesiense* to cause human sleeping sickness.

BMG-O-06
Comparative transcriptomics of Opisthorchiidae liver flukes: identification of potential therapeutic targets in detoxification system

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Introduction: Detoxification system of the parasites is potentially important for the adaptation to host environment and survival of the parasite. The studies of detoxification system in epidemiologically significant Opisthorchiidae liver flukes will help to reveal its biochemical function in parasites, and to evaluate its potential as molecular therapeutic targets in the treatment of parasitic diseases.
Materials & Methods: HiSeq 2000 sequencing; RNA-seq data analysis; Spaln2 splice-aware aligner; DESeq2, "drc", and "mortality" R packages; recombinant protein expression; UV-visible spectroscopy; drug testing *in vitro*; worm motility tests

ABSTRACTS

Cell biology and signaling

CBS-O-01

A ligand switch in the unusual *Trypanosoma* Protein Kinase A suggests a novel second messenger pathway

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Protein kinase A (PKA) is an essential and conserved signaling protein throughout the eukaryotic kingdom, including kinetoplastid parasites. In *T. brucei* PKA is activated by environmental cues like a drop in ambient temperature (cold shock), an established trigger of parasite differentiation from the mammalian to the insect stage. In contrast, *T. brucei* PKA does not bind and is not activated by the universal second messenger cyclic AMP (cAMP) that regulates PKAs of all unicellular and multicellular organisms reported to date. Here we present the biochemical and structural basis of evolutionary repurposing of a signaling protein in a phylogenetically distant parasite. First, a compound screen *in vivo* and subsequent chemical modification provided membrane permeable 7-deazapurine derivatives as activators with nM potency and exclusive specificity for parasite PKA. Candidates for an alternative second messenger *in vivo* in *T. brucei* were identified that also bind and activate in the nM range, as determined by isothermal titration calorimetry and kinase assays with purified PKA. The amazing "ligand switch" renders the parasite PKA unique and a promising target for drug development. The structural basis of this "ligand switch" was elucidated by crystal and co-crystal structures and site-directed mutagenesis. Sequence feature comparison of PKA orthologues in related kinetoplastids and our crystal structures of the *Trypanosoma cruzi* PKA suggest that the "ligand switch" occurred early in the *Euglenozoan* lineage. The novel activators were used as tools to explore for the first time downstream targets of the unusual PKA signaling in *T. brucei,* using quantitative proteomics.

CBS-O-02

Molecular and physiological analysis of social behaviour in *Trypanosoma brucei*

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Second messenger-mediated signal transduction pathways are essential for sensing and responding to environmental changes. In *Trypanosoma brucei*, a unicellular eukaryotic pathogen that causes fatal tropical diseases in humans and livestock, signaling by the second messenger cAMP is important for proper growth and cell division as well as for host-parasite interaction. The second messenger acts via novel cAMP response proteins (CARPs), most of them being unique to trypanosomes. Recently, cAMP

has been implicated in the regulation of swarming behavior (social motility (SoMo)) in *T. brucei* when cultivated on semisolid agarose surfaces, although the physiological relevance of this process in the parasite's life cycle is not yet fully understood. Here, we present the identification and characterization of novel proteins essential for SoMo and provide evidence for a role of SoMo in colonization of the insect vector. We show two-color super-resolution colocalization data for known and novel SoMo components in the trypanosome flagellum using photoactivated localization microscopy (PALM). Moreover, we specifically target a novel cAMP FRET sensor to different subcellular/subflagellar compartments in *T. brucei* in order to analyze and compare for the first time cAMP dynamics in these different compartments in liquid culture and during SoMo.

CBS-O-03
Impact of HSP90 Phosphorylation on the Life Cycle Stages of *Leishmania donovani*

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The lack of conventional gene regulation in the protozoan parasites of the genus *Leishmania* necessitates a control of protein activity on the post-translational level, e.g. by life cycle stage-dependent protein phosphorylation. In the pathogenic, mammalian stage of *Leishmania* parasites, the amastigote, parasite heat shock proteins show increased phosphorylation, indicating a role in stage-specific signal transduction. This prompted us to identify and investigate the impact of protein phosphorylation sites in the *L. donovani* heat shock protein 90. Using a chemical knock-down approach combined with genetic complementation we mutated a total of 11 confirmed or suspected phosphorylation sites and assessed the impact of such mutations on overall fitness and *in vitro* infectivity. While a number of putative phosphorylation sites were essential to maintain growth and morphology of promastigotes, at least one phosphorylation site had a selective impact on intracellular survival of amastigotes. The data presented confirm the suspected involvement of signal transduction events in the regulation of *Leishmania* HSP90 and parasite virulence.

CBS-O-04
Towards understanding how Plasmodium Sporozoites are formed

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Plasmodium sporozoites are the highly motile stages of the malaria-causing parasites transmitted by insect vectors. We use the rodent model organism Plasmodium berghei to gain insights into how sporozoites are formed and emerge from the oocysts (Klug and Frischknecht 2017). Sporozoites form within parasitic oocysts at the midgut wall of mosquitoes by a complex process involving massive

genome replication, membrane retraction and parasite budding. How his is coordinated is not clear but it was speculated that the 16 cytoplasmic microtubules play a key role in the process. These microtubules are formed at the front of the emerging parasite and arranged in a polarized manner (Kudryashev et al., 2012). We present data using electron and fluorescence microscopy showing that the deletion of microtubules does not inhibit genome replication, nuclear division, membrane retraction and the onset of budding. Yet parasites do not form due to malformations during sporozoite budding. We generated parasite lines expressing different numbers of microtubules and could correlate the microtubule numbers with defects during formation.

References:

Klug and Frischknecht, Motility precedes egress of malaria parasites from oocysts, eLife, 24;6: e19157.
Kudryashev et al., Structural basis for chirality and directional motility of Plasmodium sporozoites, Cellular Microbiology, 14:1757-68, 2012

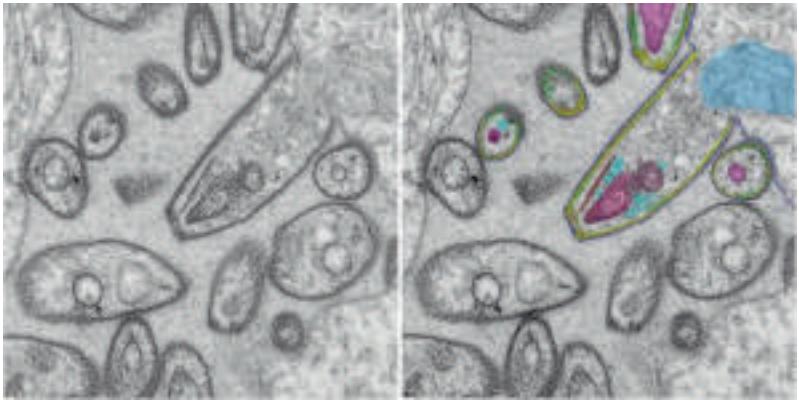

Figure 1

CBS-O-05

A SMYD3 Histone-Lysine N-Methyltransferase Homologue Regulates Life Cycle Progression in *Plasmodium* Parasite

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Despite global effort and significant progress in the last decade to treat, prevent and eradicate malaria, infections with *Plasmodium* parasites still represent a major threat to human health and a burden for global health management. Throughout their complex life cycle, alternating between a mammalian host and a mosquito vector, *Plasmodium* parasites need to tightly regulate their gene expression. In the absence of most canonical transcription factor families, epigenetic regulation has

long been recognised as a major determinant of transcriptional activity in *Plasmodium*. This includes the covalent modification of N-terminal histone tails, mainly methylation and acetylation, which regulates chromatin accessibility and gene expression. However, only few of the possible epigenetic players have been characterised in detail. Here, we describe a comprehensive knock-out (KO) screen of all histone lysine methyl transferases (HKMTs) encoded in the *P. berghei* genome. Successfully generated KO lines were phenotypically characterised throughout the entire *Plasmodium* life cycle for effects on growth within the mammalian host, gametocytogenesis, ookinete development and mosquito transmission. This led to the identification of SET6 as a regulator of parasite growth during blood-stage development within the mammalian host. SET6 is a homologue of the mammalian histone-lysine N-methyltransferase SMYD3 and parasites deficient for SET6 required significant more time to complete their life cycle. To further characterise SET6, its interaction partners and its role during *P. berghei* development, we employed biochemical as well as proteomic and transcriptomic based techniques. Taken together, we will describe the first comprehensive KO-screen of all HKMTs present in *P. berghei*, covering not only the mammalian host but also the mosquito vector.

CBS-O-06
Deciphering the regulation of the mayor S-phase promoting factor *Plasmodium falciparum* CRK4

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The *Plasmodium* life cycle consists of alternating invasive and replicative stages, both of which evolved a unique biology to support the parasitic life style. In the pathogenic blood stage of infection, *Plasmodium* parasites replicate through schizogony: consecutive rounds of DNA replication and nuclear division occur to form a multinucleated cell, before daughter cells assemble. Typically, nuclei divide synchronously when they reside in a shared cytoplasm due to the action of cytoplasmic cyclins and cyclin-dependent kinases. In a *Plasmodium* schizont, however, nuclei divide asynchronously despite sharing the same cytoplasm. Employing the inducible destabilization domain system in a loss-of-function knockdown screen, we identified the *P. falciparum* cdc2-related protein kinase (CRK) 4 as essential for proliferation in the blood stage. Knockdown of *P. falciparum* CRK4 led to a profound inhibition in the first and subsequent rounds of DNA replication. Quantitative phosphoproteomic profiling identified key effects on pathways that co-ordinate cell-cycle events including the initiation of DNA replication, histone modifications, and regulation of gene transcription. However, it remains unclear how the activity of *P. falciparum* CRK4 is regulated. *P. falciparum* CRK4 possessed at least 14 phosphorylation sites, indicating that phosphorylation plays an important role in regulation. To identify potential regulators of *P. falciparum* CRK4, we are using proximity-based labeling approaches. In parallel, we are mutating individual phosphorylation sites to gain information on their relative importance. A profound understanding of the regulatory mechanisms that orchestrate parasite replication will help identifying new intervention strategies for chemotherapeutic targeting of *P. falciparum*.

CBS-O-07

Cooperative integrin/SmVKR-1 signaling controls cell survival in the ovary of paired *Schistosoma mansoni* females

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Molecular studies in *S. mansoni* provided evidence that signaling pathways control proliferation and differentiation of the female gonad following pairing. Next to cellular tyrosine kinases (CTKs), upstream-interacting transmembrane receptors were identified such as the venus kinase receptor SmVKR1 and the beta-integrin receptor Smβ-Int1. Both receptors were found to co-localize in the ovary, and knock-down by RNAi showed comparable phenotypes exhibiting their importance for gonad differentiation, especially in the ovary. Based on these findings, we hypothesized that Smβ-Int1 may cooperate with SmVKR1 thus integrating environmental signals to regulate signalling in the ovary. Therefore, we cloned and characterized SmILK, SmPINCH, and SmNck2, molecules postulated to mediate Smβ-Int1/SmVKR1 cooperation. Besides co-localization in the ovary, all molecules were expressed in *Xenopus* oocytes. Here germinal vesicle breakdown (GVBD) was induced only if all members were co-expressed. Co-immunoprecipitation confirmed formation of a Smβ-Int1-SmILK-SmPINCH-SmNck2-SmVKR1 complex leading to SmVKR1 phosphorylation and activation in the absence of a ligand. RNAi and inhibitor studies to knock-down SmILK as a representative complex member revealed effects on the extracellular matrix around the ovary, oocyte localization within the ovary, oocyte survival, and egg production. By TUNEL assays, confocal microscopy, caspase-3 activity profiling, and qPCR analyses focusing on the pro-apoptotic genes BAK and BAX we finally showed that Smβ-Int1/SmVKR1 controls cell-death processes in oocytes. These results strongly suggest that Smβ-Int1/SmVKR1 complex formation in the ovary is important for both the maintenance of the differentiation status of oocytes and their survival. With respect to the unusual reproductive biology of schistosomes, these data also suggest that one role of the males is to prevent apoptotic processes in the female gonads during the constant pairing contact.

CBS-O-08

A functional wnt signaling pathway is essential for Echinococcus multilocularis larval development

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Introduction: The metacestode larva of the cestode E. multilocularis is the causative agent of alveolar echinococcosis (AE), which is characterized by tumor-like growth of the parasite vesicles within host organs. So far it is unknown how E. multilocularis achieves tumor-like growth but previous studies of our group showed that parasite proliferation is driven by totipotent somatic stem cells and that metacestode tissue over-expresses wnt (wingless-related) signaling components.
Methods: To further study the role of wnt-signaling in the parasite, we herein made use of Echinococcus genome information, parasite in vitro cultivation systems, newly developed RNA-

interference protocols for gene knock-down, and inhibitor assays. Results: By in silico searches for wnt signaling components present in the E. multilocularis genome we identified three orthologs of glycogen synthase kinase, two axins, and three beta-catenin orthologs. Yeast two hybrid analyses identified one of these, bcat1, as the central regulator of Echinococcus wnt-signalling. RNAi knock-down of bcat1 completely abolished the generation of metacestode vesicles from parasite stem cells in vitro. Instead of producing mature vesicles, bcat1 RNAi- cells produced immature cavities without tegument, yielded distorted muscle tissue, and an over-proliferation of stem cells. The inability of bcat1-RNAi parasite cells to produce tegument was also supported by transcriptome data. Interestingly, the general inhibition of wnt signaling in mature metacestode vesicles resulted in stem cell over-proliferation and the generation of tumor-like structures in the parasite.
Conclusions: Our data clearly indicate a decisive role of wnt signaling in the development of E. multilocularis larvae and support the hypothesis that the metacestode is posteriorized tissue. Furthermore, these data are highly relevant for the development of novel drugs against AE and for understanding proliferation and differentiation processes of Echinococcus stem cells.

Cells biology and signaling & host parasite interaction

CBH-O-01

Co-transcriptional nuclear export of mRNAs in trypanosomes

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Trypanosomes transcribe mRNAs polycistronically. Processing occurs by trans-splicing a 39 nts capped spliced leader RNA to each individual mRNA molecule, coupled to polyadenylation of the upstream gene. Trypanosomes lack homologues to most proteins that control mRNA export in yeast, have very symmetrical nuclear pores and unspliced mRNAs are detectable in the cytoplasm. Thus, trypanosomes may have either no quality control system that prevents nuclear export of unprocessed mRNAs, or the system is not tight.
We visualised mRNAs during nuclear export by multi-colour smFISH on a single, very large mRNA reporter and found that nuclear export does not require transcription to be completed. Furthermore, we have identified many proteins with a putative role in nuclear export control by proteome comparison of nuclei purified from trypanosomes in the presence and absence of trans-splicing. The majority of these proteins localises to a trypanosome-unique granule structure at the cytoplasmic site of the nuclear pores.
Taken together, our data indicate that nuclear export in trypanosomes occurs co-transcriptionally and that therefore at least the start of mRNA export does not depend on correct processing of the mRNA. Models of nuclear export control consistent with the data are discussed.

CBH-O-02
Good MORNing: new insights into the structure and function of a MORN repeat protein from *Trypanosoma brucei*

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Question: MORN repeat proteins are ubiquitous in the tree of life, but their function remains unclear. TbMORN1, a MORN repeat protein from Trypanosoma brucei, is providing new insights into the structure and function of this class of proteins.
Methods: molecular cell biology, in vitro biochemistry, protein-lipid interactions, x-ray crystallography.
Results: Depletion of TbMORN1 by RNAi is lethal in bloodstream form T. brucei. Overexpression of TbMORN1 in bloodstream form T. brucei appears to recapitulate the RNAi phenotype. The high-resolution crystal structure of TbMORN1 provides insights into its self-association. TbMORN1 can directly interact with membrane phospholipids.
Conclusions: TbMORN1 is the very first MORN repeat protein for which a high-resolution crystal structure is now available, and represents a breakthrough in understanding this ubiquitous class of proteins. Both in vitro biochemistry and cell biology strongly suggest that TbMORN1 can directly interact with membrane phospholipids, which has implications for the function of the protein, the cytoskeleton-associated macromolecular complex in which it is present, and possibly MORN repeat proteins in general. The presentation is composed almost entirely of unpublished data.

CBH-O-03
Patatin-like phospholipases of the human malaria parasite *Plasmodium falciparum*

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Introduction: *Plasmodium* parasites display a well-regulated lipid-metabolism to meet the requirements needed for their life-cycle progression, especially for the rapid erythrocytic schizogonies. While enzymes involved in the fine-tuning of lipid metabolic pathways have extensively been studied in the past, the role of phospholipases as virulence factors have been largely neglected. Phospholipases are crucial for membrane dynamics during host cell infection and egress as well as for replication and cell signaling, as it has been shown for other intracellular pathogens.
Objective: The objective of this study was to functionally characterize the four patatin-like phospholipases PLPL1-4 in the *P. falciparum* blood stages with special regard to proliferation, egress and transmission of the malaria parasite.
Material & Methods: For expression and localization studies, we performed semi-quantitative RT-PCR and immunofluorescence analyses. For functional characterization, we generated inducible knockdown mutants using the ribozyme-based mRNA-degrading glmS-system. Loss-of-function

phenotypes after conditional depletion of the phospholipases and the effect of phospholipase inhibitors were investigated in growth, gametocyte development and exflagellation assays.
Results: We here show the first comprehensive bioinformatic analysis of the plasmodial phospholipases with particular focus on PLPL1-4. RT-PCR revealed transcript expression of the PLPL1-4 in all blood stages. Immunofluorescence assays demonstrated a cytosolic vesicular expression pattern both in asexual and sexual blood stages. PLPL1 and PLPL2 co-localize with microneme-resident protein AMA-1. We further provide an analysis of the different growth phenotypes after conditional depletion of the phospholipases.
Conclusion: We hypothesize that plasmodial phospholipases are crucial for parasite proliferation and transmission and therefore might be effective targets for future chemotherapeutics.

CBH-O-04
An exclusive guanylate cyclase governs the lytic cycle of *Toxoplasma gondii*

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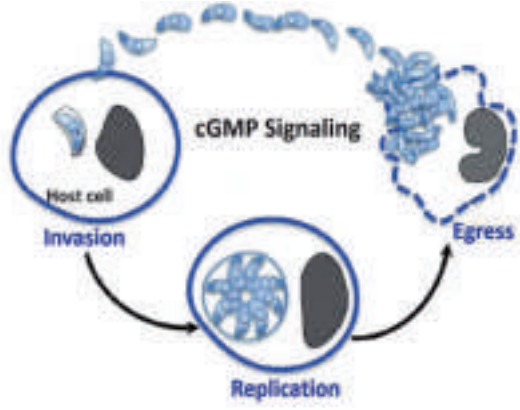
Introduction: *T. gondii* is an opportunistic intracellular parasite causing life-threatening diseases in immuno-immature and immunocompromised individuals. Its asexual reproduction in mammalian host cell is mediated by cGMP signaling.
Objectives: We aimed to determine the physiological importance of the guanylate cyclase (*Tg*GC) and corresponding protein kinase G (*Tg*PKG) for the lytic cycle of *T. gondii*.
Materials and Methods: Biochemical, cell biological and reverse genetics approaches were used to characterize *Tg*GC and *Tg*PKG. 3´-insertional tagging of both genes was performed to determine the native expression and subcellular localizations. A knockdown of *Tg*GC and *Tg*PKG by Cre-recombinase mediated excision of the 3" UTR was done. Growth phenotyping was conducted to ascertain the possible effects of genetic knockdown on individual steps of the lytic cycle.
Results: *Tg*GC is an unusual protein, which has four predicted P-Type ATPase domain at the N-terminal, and two nucleotide cyclase domains at the C-terminal end. The epitope-tagged *Tg*GC-HA localizes predominantly at the apical end, whereas *Tg*PKG-HA is distributed in the cytomembranes. Transcriptional destabilization of *Tg*GC and *Tg*PKG proteins caused a corresponding inhibition of the parasite growth (~40%) in plaque assay. The invasion rates of both mutants were impaired by 30-40%. Motile fraction of mutants was reduced by half, and the average trail length was remarkably shorter (~20 µm) than the parental strain (~55 µm). Although an apparent delay in egress was observed 40hpi, *Tg*GC-KD showed a normal egress in late (64hpi) cultures. Treatment of BIPPO and Zaprinast (cGMP-dependent phosphodiesterase inhibitors) completely rescued the phenotypic defects in the *Tg*GC mutant, while Compound2 (PKG inhibitor) almost blocked the parasite growth.
Conclusion: Lytic cycle *of T. gondii* is regulated by cGMP signaling, which is commenced by an exclusive GC and transduced by a dedicated PKG.

Figure Legend: The motility, invasion and egress of tachyzoites is regulated by cGMP signaling during the lytic cycle of *T. gondii*.

Figure 1



CBH-O-05

Binding of host C-type lectin receptors to *Toxocara* spp.-derived ligands

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The dog roundworm *Toxocara canis* and the cat roundworm *Toxocara cati* are worldwide distributed zoonotic gastrointestinal helminths with a frequent exposure to humans. Within paratenic host's tissue, third stage larvae can persist up to a decade by immune evasion and cause severe disease pathology such as neurotoxocarosis and ocular *larva migrans*. Upon infection, the initial recognition of pathogens is mediated by pattern recognition receptors (PRRs) in innate immunity. Myeloid C-type lectin receptors (CLRs) are PRRs initiating immune responses by the recognition of pathogen-associated molecular patterns (PAMPs). CLRs recognize carbohydrate structures of bacteria, virus, fungi and parasites often in a Ca^{2+}-dependent manner.
There is a gap of knowledge on whether and how myeloid CLRs recognize *Toxocara*-derived ligands. Therefore, binding of host CLRs to *Toxocara* spp. somatic- and ES-antigen was evaluated by the use of a comprehensive CLR-Fc fusion protein library. In ELISA-based assays, *T. canis* and *T. cati* somatic- as well as ES-antigen was bound by several CLRs such as Mincle, Dectin-1, DC-SIGN and MGL-1, highlighting a potential role of these CLRs in *Toxocara* infection. *T. canis* and *T. cati* exhibited equal binding patterns indicating a similar composition of ES- and somatic antigen in both species. Binding studies of CLRs to third stage larvae using fluorescence microscopy is currently ongoing. To elucidate the impact of CLRs on dendritic cell (DC) maturation and effector functions during toxocarosis, DC stimulation studies are performed. To this end, DCs derived from selected CLR-deficient mice are stimulated with ES- and somatic antigen with subsequent assessment of effector cytokines.

In conclusion, CLRs are promising candidates for immune modulation during *Toxocara* infection as indicated by the specific binding of host CLRs to *Toxocara*-derived antigens.

CBH-O-06
Starving a deadly parasite: *In vitro* metabolomic studies on the *Echinococcus multilocularis* metacestode.

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The metacestode (larval stage) of the tapeworm *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE), which is a severe and in many cases incurable disease in humans and other mammals. Livelong benzimidazole chemotherapy is often the only option for AE patients. Benzimidazoles can cause substantial side effects and therefore novel therapeutic treatment strategies are urgently needed.
We follow the strategy to discover new treatment options against AE by investigating the host-dependent nutritional requirements of the parasite. Like this, specific factors could be targeted to starve the parasite within the host. We applied metabolomic profiling by 1H Nuclear Magnetic Resonance (NMR) spectroscopy to investigate the nutritional requirements of the *E. multilocularis* metacestode in an *in vitro* model. Of the unambiguously detected metabolites, six were significantly consumed from the medium and thirteen released into the medium. Several released metabolites (including succinate, malate, and fumarate) are involved in the anaerobic malate dismutation pathway, which could offer a potential drug target, as it is not found in mammals. Among the most consumed metabolites was the amino acid threonine. Preliminary experiments showed increased parasite respiration upon addition of threonine to *E. multilocularis* metacestodes, which indicates that threonine can be used as a substrate for the energy metabolism of *Echinococcus*. Currently, we confirm the above-described NMR results by amino acid quantification assays, and specific measurements of the energy metabolism and metabolic products. In the long-term, we aim to compare these *in vitro* findings with the *in vivo* mouse model in order to identify innovative ways for starving the parasite.

Focus session • Compartments of intracellular parasites

CIP-O-01
Understanding gliding motility of the malaria parasite through proteomics approaches

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Life cycle progession of the malaria parasite *Plasmodium* is highly dependend on gliding motility of two motile stages, ookinetes and sporozoites. Ookinetes need to be motile in order to penetrate the midgut epithelium were they establish an infection at the gut wall to develop into hundreds of sporozoites. Sporozoites need to be motile to enter salivary glands of the mosquito and to actively move in the skin of the host to find a blood vessel. Adhesion and motility of both cell types rely on the secretion of proteins at the apical end from specialized organelles called micronemes. In order to identify unknown proteins with a potential to be important during parasite motility we have defined the microneme content (microsome, 143 proteins) of ookinetes of the rodent model organism

Plasmodium berghei using proximity dependent biotinylation (BioID). Furthermore we identified 161 proteins secreted by sporozoites. We now use gene deletion of selected candidates and identified essential functions for 2 proteins. In conclusion, we could for the first time apply BioID to mosquito stage parasites and together with our sporozoite secretome provide a set of novel proteins in two different stages important to gain a better understanding of gliding motility and parasite transmission.

CIP-O-02

The protozoan Toxoplasma gondii uses a multi-functional device that mechanically mimics dynamins to give birth to a unique parasitophorous vacuole prone to rapid remodelling

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Question: The obligate intracellular protozoan parasite *Toxoplasma gondii* injects a multi-unit nanodevice into the host cell Plasma Membrane (PM) that connects the two cells through a circular Tight Junction (TJ), and directs the folding of an unconventional microbial entry vesicle. When entry is about complete, the adjacent bilayer regions that connect the budding vesicle to the PM merge and are severed, thus accounting for the release in the host cell cytoplasm of a sealed Parasitophorous Vacuole (PV) that houses the parasite and supports its growth. How the PM-derived bud seals and pinches off a PV is poorly understood signifying that the mechanism of these crucial steps to access host cells remains largely elusive.
Methods: We applied high-speed quantitative live fluorescence imaging, kinematic and mathematical modeling, to monitor in real time the process of *Toxoplasma* PV formation. Specific tagging of the core component of the invasive device used by the *Toxoplasma* tachyzoite stage together with a set of host cell PM markers allowed precise visualization of the whole entry process including PM invagination, vesicle budding and scission as well as early PVM behavior.
Results: We report that the tachyzoite nanodevice mechanically promotes membrane scission at the TJ-PM interface, allowing birth of the sealed Parasitophorous Vacuole (PV) in the host cell cytoplasm. While a specific twisting motion of the parasite drives asymmetric distribution of forces to close the device at the TJ, interfering with the process induces irreversible osmotic damages to the parasite. When the device closes properly, membrane fission and PV cytoplasmic traveling are revealed by two distinct motion sequences of specific kinematical parameters.
Conclusions: We propose that, in addition to functionally mimicking host cell dynamins and accounting for PV birth, device closure sets *T. gondii* for rapid adjustment to intracellular lifestyle through the early burst of PV membrane remodelling following the twisting motion.

CIP-O-03

The role of host factors in *P. falciparum*-mediated host erythrocyte modification

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The malaria parasite *P. falciparum* invades and replicates within human erythrocytes. The parasite massively modifies the biochemical and biophysical properties of this cell, mediated by parasite proteins that are exported to the host cell. How the parasite exports proteins to the host cell has been

the focus of intensive research over the past decade, and has revealed several novel protein trafficking signals and mechanisms, including a protein translocase, PTEX, which is required for protein export across the parasitophorous vacuolar membrane. To pass through PTEX, proteins must be held in a translocation-competent state, and it is expected that such proteins must then re-fold. However, the molecules involved in this process have remained, until now, elusive. Here we address this biological problem using an optimized permeabilised cell system. Our data suggest that protein translocation into the host cell requires ATP, is insensitive to inhibitors of Hsp90, and is sensitive to protease treatment. We then studied a potential role for human Hsp70 (HsHsp70) in protein trafficking. Cell fractionation suggested that HsHsp70 is found in an ATP sensitive membrane-bound form at the trans-side of the PVM. Using recombinant wild-type (WT) and dominant-negative (DN) human Hsp70 paired to our permeabilised cell system, we demonstrate that functional HsHsp70 is required for protein translocation. Finally, by incorporation of both WT and DN HsHsp70 into resealed erythrocytes, we show that HsHsp70 is essential for parasite growth. Our study reveals a fascinating interplay of parasite and host factors at the parasite-host interface.

CIP-O-04
Unraveling the function of EXP1 in the asexual development of *P. falciparum* parasites

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Malaria parasites propagate within a compartment termed parasitophorous vacuole (PV) isolated from the red blood cell (RBC) cytosol by the PV membrane (PVM). The PVM is the interface between the parasite and the host RBC with vital roles for parasite survival. EXP1 is a PVM integral protein that is highly expressed in blood and liver stages with likely essential functions. Previous work proposed that EXP1 is a glutathione S-transferase (GST) that protects the parasite from oxidative damage caused by products of the hemoglobin metabolism. Nevertheless, there is so far no direct functional data on EXP1 in *P. falciparum* parasites.
To unravel the function of EXP1 we used selection linked integration to generate a knock-out cell line where the *exp1* gene can be conditionally excised via DiCre recombinase in a rapalog inducible manner. Parasites without EXP1 (ΔEXP1) displayed a severe growth defect, leading to an accumulation of aberrant trophozoites and picnotic parasites which did not complete the schizogony.
Functional assays showed that protein export, RBC modifications and hemoglobin uptake were not impaired after depletion of EXP1. Interestingly, while ΔEXP1 parasites possessed an intact PVM, loss of EXP1 affected the organization of the vacuolar compartment and the distribution of PV proteins. Time lapse imaging revealed that ΔEXP1 parasites show aberrant behavior, morphology and positioning in the host RBC.
Furthermore, we established a genetic complementation approach with several mutated and deletion EXP1 constructs. Our results indicate that important EXP1 functions reside in the transmembrane domain and the N- and C-terminus of the protein. In contrast, the proposed GST activity is partially dispensable for the development of asexual blood stages, since constructs with mutations of the putative GST catalytic site restored parasite growth nearly to the same extent than the wild type construct. Consistently, ΔEXP1 parasites were not under elevated oxidative stress and were not rescued by reducing agents.

Taken together, these results demonstrate that EXP1 indeed is essential for the propagation of *P. falciparum* asexual blood stages and our results point to a role related to the maintenance of the PV rather than a function as GST.

CIP-O-05

A stress granule-resident seven-helix protein regulates translation during transmission of the malaria parasite *Plasmodium falciparum*

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One of the major challenges during life-cycle progression of the malaria parasite *Plasmodium falciparum* is the human-to-mosquito transmission, which starts with the differentiation of sexual precursor cells, the gametocytes. Once taken up by the blood-feeding *Anopheles* vector, these rapidly convert into gametes, a process accompanied by high protein synthesis rates. We aimed to elucidate the role of a putative seven helix protein, termed 7-Helix-1, during parasite gametogenesis and sexual reproduction and to investigate its function in protein synthesis.
For expression and localization studies, we performed IFA and mRNA-FISH-IFA experiments. Further, stress granule core fraction enrichments followed by Western blots were conducted. Using reverse genetics we generated a 7-Helix-1-KO line and analysed its phenotype in transmission assays and *in vitro* assays. Microarray studies were performed in order to investigate differences in transcript abundance. For the analysis of protein interactions co-immunoprecipitations have been carried out.
7-Helix-1, a homolog of human LanC-like 2, accumulates in stress granules of female gametocytes. Malaria parasites lacking 7-Helix-1 are significantly impaired in female gametogenesis and thus transmission to the mosquito. Lack of 7-Helix-1 further leads to a deregulation of components required for protein synthesis. Consistently, inhibitors of translation could mimic the 7-Helix-1 loss-of-function phenotype. The RNA-binding protein Puf2, known to regulate translation of the female-specific antigen Pfs25, was identified as an interaction partner of 7-Helix-1. In accord, lack of 7-Helix-1 leads to impaired Pfs25 synthesis. Our data demonstrate that 7-Helix-1 is a component of stress granules and here involved in regulating translation of proteins needed for parasite development in the mosquito.

CIP-O-06
A novel *Plasmodium* protein and its critical function for host-to-vector transmission

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Ferlins form an ancient protein family with essential functions and are present in most eukaryotes. The founding member of this family is a fertility factor acting in the process of spermatogenesis in the nematode *C. elegans*. Ferlins typically mediate vesicular fusion in a calcium-dependent manner. Formation of microgametes (motile male gametes) in *Plasmodium* is known to be critically dependent on both calcium signalling and the discharge of specialized vesicles. We have achieved a stage-specific depletion of the essential ferlin-like-protein (FLP, a ferlin family member) in the gametocyte stage by exchanging its promoter. The parasite line in which FLP is under the control of the PBANKA_140060 promoter (Δflp_{gam}), shows normal development in asexual blood stages and gametocyte production. Following activation, Δflp_{gam} male gametocytes fail to produce freely moving microgametes. Instead, microgametes remain trapped within the host erythrocyte and form a bundled motile unit. This failure of egress results in a complete arrest of the life cycle, as subsequent life cycle stages could not be observed *in vitro* or *in vivo*. In line with the observed egress failure, the phenotype could be rescued by chemical membrane lysis *in vitro*. These data show that FLP plays an essential role during host-to-vector transmission, specifically in the process of microgamete egress. In agreement with the vesicular localization typical for ferlins, endogenously HA-tagged version of FLP localizes to small speckles, which relocalize towards the cell periphery after gametocyte activation. We propose that FLP might act as a *Plasmodium* fertility factor, linking the calcium signalling and discharge of vesicles during the process of gametogenesis.

DDD Symposium

DDD-O-01
Evaluation of the Pharmacokinetic-Pharmacodynamic Relationship of Praziquantel in the *Schistosoma mansoni* Mouse Model

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After more than 40 years of use, Praziquantel (PZQ) still remains the drug of choice for the treatment of intestinal and urogenital schistosomiasis. Its anti-parasitic activity resides primarily in the (*R*)-enantiomer. Hitherto neither the molecular target nor the pharmacokinetic-pharmacodynamic relationship have been fully elucidated. Here we investigated the efficacy and pharmacokinetics of PZQ in the *Schistosoma mansoni* mouse model to determine the key factors that drive its efficacy.

Dose-response studies with racemic PZQ with or without addition of an irreversible pan-cytochrome P450 (CYP) inhibitor, 1-aminobenzotriazole (ABT), were performed. In addition, efficacy of PZQ in the presence of the CYP inducer, dexamethasone (DEX), was determined. Plasma samples were obtained by tail vein bleeding at 4 time points. The (*R*)-PZQ levels were determined using a LC-MS/MS method. Non-compartmental pharmacokinetic analysis was performed using PKsolver. In addition, experiments using an enhanced *in vitro* assay were conducted.
We found that the use of ABT increased (*R*)-PZQ plasma exposures in the systemic circulation by ~10 to 20 fold but the latter were not predictive of efficacy. The use of DEX decreased plasma exposures of (*R*)-PZQ in the systemic circulation by ~10 fold without reducing efficacy. We extrapolated the (*R*)-PZQ concentrations in mouse portal vein / mesenteric veins from the systemic exposures and found that a free exposure of (*R*)-PZQ of ~ 20 µM*h in the portal vein was needed to obtain a worm burden reduction >60%.
It is suggested that the high (*R*)-PZQ concentrations available before the hepatic first pass metabolism drive the efficacy against *S. mansoni* adult worms residing in the mesenteric veins. It is then possible that the current dosing regimen of 40 mg/kg in preventive chemotherapy programs may provide suboptimal concentrations in low-weight patients such as children, due to smaller total amounts of drug administered, and may consequently result in lower cure rates.

DDD-O-02
Effect of arthropod antimicrobial peptides againist *Plasmodium falciparum*

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Malaria is a mosquito-borne disease affecting millions of people mainly in Sub-Saharan Africa, Asia and some South American countries. Drug resistance to first-line antimalarial drugs (e.g. chloroquine, artemisinin) is a major constrain in malaria control. Antimicrobial peptides (AMPs) have shown promising results in controlling *Plasmodium* spp. parasitemia in *in vitro* and *in vivo* infection models. AMPs are important components of the innate immunity of invertebrates and vertebrates. Currently, it is widely recognised that many organisms use AMPs as a defense system against microbial infection. They have broad spectrum antimicrobial activity against bacteria, fungi and viruses. The potential activity of AMPs against protozoan parasites is less known. In this study we tested in total 15 AMPs from arthropods, 10 AMPs from three different insects; drosocin, two metchnikowins (*Drosophila melanogaster*), stomoxyn, LSerPRP-2, LSerPRP-3 (*Lucilia sericata*), four cecropins A, B, C, D (*Galleria mellonella*) and five tick defensins DefMT2 – DefMT7 (*Ixodes ricinus*) against the protozoan parasite *Plasmodium falciparum*. Two metchnikowins and four tick defensins inhibited *P. falciparum* growth *in vitro*. We further tested the antiplasmodial activity of three of the tick defensins in a mouse model of malaria. Defensin treatment inhibited significantly the replication of *Plasmodium chabaudi*. Furthermore, defensin injection was not associated with red blood cell hemolysis. These findings justify further studies on the use of insect AMPs to control malaria.

DDD-O-03
Alive and green: Refining Chagas' disease *in vitro* drug screening

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One of the major characteristics of Chagas' disease – caused by *Trypanosoma cruzi* – is a decade-long gap between the phases of acute and chronic disease. During this time, termed indetermined chronic Chagas' disease, the parasite is mostly not detectable. Yet, the combination of parasite replication and the immune response cause the chronic disease symptoms. The parasite presence has been linked to disease severity, rekindling the search for trypanocidal drugs. The high rate of relapses of posaconazole-treated patients in the phase II trial (1) has spotlighted the difficulties, which the *in vitro* and *in vivo* drug testing systems of the day had to determine the cidality profile of a candidate. A lot has changed since: State of the art *in vitro* assays employ fluorescence-based high-content microscopy and computer analysis improving the detection limits among other features. Yet, parasite quantification is mostly based on fluorescent nuclear staining requiring fixation.
We have established a green fluorescent *T. cruzi* line for the use in *in vitro* assays. GFP is expressed cytosolically in the major life cycle stages. Therefore, it is possible to image the living parasites with a high-content microscope. The computer program based analysis of time-lapse images evaluates the growth pattern of the parasites under the influence of drug candidates. This allows conclusions on the drug action, such as the time-to-kill and the difference between being cidal and static drugs. Furthermore, in wash-out assays, the same parasites can be imaged over a period of weeks repeatedly in order to determine the time to relapse.
Employing a green-flourescent *T. cruzi* parasite line enables us to refine time-to-kill and wash-out assays. These *in vitro* assays will help to select a trypanocidal drug to prevent chronic Chagas' disease symptoms.

1) Molina et al. (2014) Randomized Trial of Posaconazole and Benznidazole for Chronic Chagas' Disease. DOI: 10.1056/NEJMoa1313122

DDD-O-04
***Echinococcus multilocularis*: from drug screenings to the energy metabolism**

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Alveolar Echinococcosis (AE) is a lethal parasitic disease in humans. Its etiological agent is the fox tapeworm *Echinococcus multilocularis*, which can be found across the globe in the Northern hemisphere. Metacestodes (secondary larva) of the parasite grow infiltrative in the liver and can also metastasize into distal organs. Thus, AE has many pathological similarities with a malignant hepatic

tumor. Current chemotherapeutic treatment against AE consists of the benzimidazoles albendazole and mebendazole. However, they are merely parasitostatic and can have serious side effects. Therefore, new treatment options against AE are urgently needed.
Our aim is to find and explore new active compounds against *E. multilocularis*. The Medicines for Malaria Venture (MMV) pathogen box is a library of 400 diverse molecules active against a wide range of pathogens. We screened the MMV pathogen box against *in vitro* cultivated metacestodes of *E. multilocularis* and assessed drug-induced damage using the PGI assay. We found 13 promising compounds that were active at 10µM. Moreover, four of these drugs showed activity at 1µM, and their IC50 values were assessed along with their cytotoxicity against two different mammalian cell lines. One compound (MMV689480, Buparvaquone) was particularly promising because it is already clinically applied against theileriosis in cattle. It was further tested in experimentally infected mice, where it failed to be active. Therefore, current experiments focus on the mode of action of Buparvaquone in order to understand its lacking *in vivo* activity, with a particular focus on the energy metabolism of *Echinococcus*.

DDD-O-05
Pharmacokinetics, efficacy and safety of ascending dosages of ivermectin against *Trichuris trichiura* in preschool- and school-aged children

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Introduction: Ivermectin exhibits a broad activity against parasitic worms, but so far it has been poorly characterized to treat *T. trichiura;* the helminthic specie with poorest medical options. The pharmacokinetic (PK) behavior and optimal dose in children infected with *T. trichiura* remains unknown and children (< 15 kg) are excluded from treatment due to missing safety information. A dose-finding and PK study was performed with 160 school-aged children (SAC) and 120 preschool-aged children (PSAC) infected with *T. trichiura* to evaluate the PK profile, safety and efficacy of ivermectin.
Methods: SAC (6–12) and PSAC (2–5) infected with *T. trichiura* were enrolled in the study in Côte d"Ivoire. SAC were treated with a single oral dose of 200, 400 or 600 µg/kg and PSAC with 100 or 200 µg/kg of ivermectin; a placebo arm served as comparator. Dried blood spots (DBS) technology was performed at 11 time points and samples were analyzed with a LC-MS/MS method that has been developed and validated prior to the trial to obtain PK parameters. The efficacy of ivermectin was evaluated by the Kato-Katz thick smear technique of stools samples collected prior and post treatment to evaluate cure (CR) and egg reduction rates (ERR). Adverse events were assessed by questionnaires before, 3, 24 and 72 hours after treatment.
Results: Preliminary PK data of SAC samples show dose-Cmax and dose-AUC correlation, while Tmax is dose independent. CRs were low in all treatment groups. The highest ERR of 66.3% was observed with 600 µg/kg. Only few, mild clinical symptoms were reported by the children.
Conclusion: The preliminary results from SAC show a dose-dependent PK profile with identical time to absorb ivermectin. The low efficacy of ivermectin against *T. trichiura* in children was not expected, but considering its good safety profile higher doses could be tested. Generally, results hint that the drug needs to be administered in combination (*i.e.,* with albendazole) to effectively treat *T. trichiura.*

DDD-O-06

Insights on the mode of action of the novel isoxazoline ectoparasiticide Lotilaner (Credelio™)

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Introduction: Drugs acting against endo- or ectoparasites are often targeting neuroreceptors. One of the most recent examples is the class of isoxazolines that is described to act as specific blocker of γ-aminobutyric acid-gated chloride channels (GABACls) and to a lesser extent of glutamate-gated chloride channels of insects. Lotilaner belongs to this class and has been registered recently in Europe as Credelio™ for use against ticks and fleas on dogs.
Objectives: The present study aims to elucidate the mode of action and the specificity of lotilaner on GABACls of insects, ticks, crustaceans and mammals.
Materials & Methods: The GABACl-genes of *Drosohpila melanogaster*, *Lepeophtheirus salmonis* (sea lice), *Rhipicephalus microplus* and *Canis lupus familiaris* (dog; Beagle) were expressed in *Xenopus* oocytes. An automated two-electrode voltage clamp electrophysiology-system was used to assess the functional impact of lotilaner on GABACls compared to the natural agonist GABA, as well as the antagonists dieldrin and fipronil.
Results: Lotilaner has been identified as a potent non-competitive antagonist of invertebrate GABACls. The antagonistic activity remained unchanged on dieldrin- or fipronil-resistant mutants, suggesting that lotilaner might bind to a site at least partly different from the one bound by known GABACl blockers. This hypothesis was supported by co-application experiments that revealed a different antagonism of lotilaner compared to fipronil. Finally, we found a high specificity of lotilaner for insect-, tick- and sea lice-GABACls, but no activity on the dog GABAA receptor.
Conclusion: The above results demonstrate that lotilaner is a non-competitive antagonist specific to GABACls of invertebrates. The mode of action seems different to other known antagonists, since it can overcome resistance to dieldrin and fipronil. Finally, the lacking activity on the mammalian receptor is supporting the favorable safety profile of lotilaner.

DDD-O-07

Ladybird beetles and schistosomes – inhibitory effects of the novel antimicrobial compound harmonine on survival and reproduction of *Schistosoma mansoni*

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Introduction: Identification of novel anti-schistosomal drugs is pressing – not only because schistosomiasis is one of the most important tropical infectious diseases worldwide, but also because of the unavailability of a vaccine, and the upcoming fear of resistance to the sole drug in use, Praziquantel.
Objectives: Harmonine is an antimicrobial compound discovered in the invasive Asian ladybird *Harmonia axyridis*. First application tests showed activity of harmonine against mycobacteria as well as *Plasmodium* and *Leishmania* parasites. To explore the potential of harmonine as a template for the development of novel antihelminthics, we tested its capacity to affect helminth parasites, specifically *Schistosoma mansoni*.
Materials & Methods: Adult *S. mansoni* couples were exposed for 72 h to harmonine at various concentrations *in vitro*. Motility, survival, egg production, and morphological phenotypes were assessed by light and confocal microscopy.
Results: Harmonine was lethal to *S. mansoni* at concentrations as low as 10 µM. With 5 µM, the motility of worms was reduced to a minimum, female and male worms separated, and egg production was fully abolished. Tegument blisters and prominent gut dilatations were observed. Confocal microscopy revealed structural disintegration of the male and female reproductive organs, including reduced sperm numbers, which might point to a cellular target of harmonine involved in gonad development or function.
Conclusion: Next to its antimicrobial and anti-protozoan effects, harmonine has also anti-schistosomal activity, which promotes this natural compound as an attractive candidate for drug development. Whether other helminth species, such as the liver fluke *Fasciola hepatica*, are also affected by harmonine is currently under investigation, as well as the identification of its cellular target.

Diagnosis & prevention; phylogeny and evolution

DPP-O-01

Establishing a protocol for simultaneous comparative molecular and proteomic species identification of individual cyathostomin worms

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Introduction: Cyathostomins are considered to be the most important equine parasites due to their high prevalence, potentially fatal larval cyathostominosis and emerging anthelmintic resistance. Currently, 50 cyathostomin species are accepted and morphological species identification of adult nematodes is limited to highly trained experts. Whereas research focuses on molecular methods to facilitate species identification, the protein profiling is a promising technique for rapid and cost-effective species identification.
Objectives: The study aims to establish concurrent molecular and proteomic approaches for cyathostomin species delineation and to set up a master-spectra library on several species of adult cyathostomins.
Materials & Methods: Adult cyathostomins were collected from the intestinal content of horses. The species and their sex were morphologically identified. For the proteomic approach, proteins were extracted with formic acid/acetonitril; the extracted proteins were spotted and mass spectrometry measurements were carried out in a broad *m/z* range (2000-20000 kDa). Subsequent to protein extraction, DNA was isolated from the buffered sediment of the homogenization followed by PCRs targeting the ITS-2 and COI gene.
Results: After protocol optimization, MALDI-TOF MS spectra for individual adult cyathostomin specimen and PCR products from the same worm were reproducibly obtained. For three morphospecies, differences suggesting the presence of cryptic species were found.
Conclusion: The complementary use of MALDI-TOF MS and PCR was positively evaluated for cyathostomin species identification. Establishing a master-spectra library for several species of cyathostomins will allow independent and consistent species identification. Furthermore, the current results confirm that the morphological identification of cyathostomin species is not sufficient. Cryptic species can be characterized by combining morphological data, protein profiling and DNA barcoding.

DPP-O-02

The application of isothermal nucleic acid amplification in diagnostics and point-of-care testing for the detection of parasites

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Introduction: The use of nucleic acid amplification techniques has significantly improved the specificity, sensitivity of diagnostic tests. However, the polymerase chain reaction (PCR), often described as the gold-standard for molecular diagnostics, usually remains laboratory-bound and is not available for on-site tests. Moreover, complex and expensive instruments are needed to conduct PCR assays. To avoid some of these obstacles, a possible alternative for the detection of pathogenic organisms could be the use of isothermal nucleic amplification.
Objectives: In the past, several assays and systems for the detection of numerous pathogenic organisms based on isothermal nucleic acid amplification have been established in our group. The great strength of alternative read-out options included naked-eye (turbidity and colorimetry), spectrometric and fluorometric formats were tested and optimized to offer maximum flexibility for system integration. Approaches using lateral-flow dipsticks as detection format offer potential apparatus free amplification and read-out.
Materials & Methods: As an example the detection of *P. falciparum* is presented. Two target specific oligonucleotide primers and one probe were designed and the reaction conditions were tested and evaluated oriented towards the potential practical application.
Results: We were able to demonstrate the specific detection of *P. falciparum* in less than 20 minutes, including the read-out, while we achieved sensitivity comparable to laboratory methods. In addition a special primer/probe – system allows the direct detection of the amplification product on lateral-flow-strips without further treatment. The reaction runs at a constant temperature of 38°C and is not sensitive to temperature variations or inhibitory substances.
Conclusion: The isothermal amplification and strip detection system can work instrument-free and the result is given in a format which can be read out by the untrained eye. This flexible system could offer a possibility for a true nucleic acid rapid-test for malaria and other pathogens in the field where trained health-care workers and medical infrastructure may not be available.

DPP-O-03
Pre-clinical evaluation of a double genetically arrested parasite (dKO GAP) for malaria vaccine development

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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 *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, *P36p(-)* sporozoites elicit long-lasting immunity, but lead to break-through infections during immunizations.
Here, we combined the two knockouts *SLARP(-)* and *P36p(-)* and present a systematic pre-clinical evaluation of this dKO GAP parasite line, which is a crucial step before translation to human clinical trials. We show complete arrest of *SLARP(-)/P36p(-)* parasites in cultured hepatoma cells and sporozoite-infected mice. We analyzed these dKO GAP sporozoites for their immunogenicity and their potential to induce protection in comparison to the respective single knockout lines as well as irradiated sporozoites (gspz). Animals immunized with *SLARP(-)/P36p(-)* parasites elicit similar antibody titres and numbers of IFNg-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 dKO GAP vaccine. dKO GAPs serve as a platform towards safer and more immunogenic whole sporozoite vaccine candidates.

DPP-O-04
Onchocerciasis Associated Epilepsy is preventable: Case of Bilomo in the Mbam Valley, Cameroon

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Background: High epilepsy prevalence has been reported in onchocerciasis (oncho) endemic areas, where a spectrum of seizure disorders including nodding syndrome (NS) can be found. These are

collectively known as onchocerciasis associated epilepsy (OAE) due to the suspected role of *O. volvulus* (Ov) in inducing these conditions particularly in the 5-18 years age group. Proper oncho control may affect epilepsy incidence in such areas.
Methods: A door-to-door household survey was carried out in July 2017 in Bilomo – an oncho endemic village with data on epilepsy prevalence prior to ivermectin (IVM) mass drug administration (MDA). Resident members of each household were screened for seizures and IVM intake. Participants with a history of seizures underwent a neurological evaluation to confirm the diagnosis of epilepsy. Data from the current study were compared with epilepsy data obtained in 1998. Anti-ov16 IgG antibodies were measured using rapid diagnostic tests in children aged 7-10 years to assess ongoing oncho transmission.
Results: 1321 individuals in 193 households were screened; mean age: 23.8±20.3 years). 61 participants (4.6%) had epilepsy including 9 (0.68%) with probable NS. 27.80% of persons with epilepsy (PWE) were aged 10-19years in 2017 compared to 60.21% in that age group in 1998 (p = 0.001). 60.70% of PWE were aged 20-34years in 2017 compared to only 34.41% in 1998 (p = 0.001). 63.17% of participants aged ≥5 years reported taking IVM at least once in the last two years. Finally, 53.10% of children < 10 years old were positive for anti-Ov16 antibodies.
Conclusions: Epilepsy prevalence did not change significantly in 2017 (4.6%) compared to 1998 (4.9%). However, a significant age shift of PWE towards the older age groups was observed as many PWE evolved from the 10-19 years in 1998 to 20-34 years in 2017. Considering a fairly similar age distribution in both years, this suggests a lower epilepsy incidence in the 10-19 age group possibly attributable to oncho control by IVM. One round of low coverage MDA annually however seems insufficient to stop oncho transmission and affect epilepsy prevalence. Strengthening oncho elimination programs with at least biannual IVM MDA and vector control measures could be the key to preventing new cases of OAE.

DPP-O-05
Evolution of surface antigens and invasion-related genes in the coccidian parasite *Cystoisospora suis*

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Introduction: Parasite genomes are shaped by host and tissue ranges, with gene loss being one of the hallmarks of parasite genome evolution. However, the basis of host/tissue specificity are still poorly understood in coccidia. Cyst-forming coccidia, such as *T. gondii* and *N. caninum* display wide host and tissue range preferences. In contrast, *C. suis* only targets intestinal epithelial cells of piglets. Surface antigens (SRS) and invasion-related genes (IRGs), such as microneme (MIC), rhoptries (ROP) and dense granules (GRA) represent the first line of the interaction with the host and might show variation in content as a consequence to single host/tissue adaptations.
Objectives: In this study, we take advantage of the recently sequenced *C. suis* genome to perform a comparative genomics study to highlight differences in gene content that might explain the phenotypic differences from other coccidia. Specifically, we tested the hypothesis that adaptation to a single host/tissue might result in loss of surface antigens or invasion-related genes.
Material and methods: We generated gene families by clustering the genes of *C. suis* and six other closely related coccidian species using the OrthoMCL tool. By a combination of text and domain-based searches, we extracted surface antigens and invasion-related genes and inferred the patterns of gene family gains/losses by Dollo parsimony.

Results: We did not find evidence for loss of SRS or IRGs in the *C. suis* lineage, rather we observed a rapid expansion of SRS, ROP and GRA genes and in the ancestor *of C. suis*, after the split from *S. neurona.* While MIC genes are overall more conserved, we detected an amplification of MIC4, which was previously found to be involved in intestinal epithelial cell attachment in *T. gondii* and might represent a candidate for tissue-specificity in *C. suis*.
Conclusion: Gene duplication of SRS and IRGs, rather than gene loss, might contribute to host and tissue specificity in *C. suis*.

DPP-O-06
Phylogeny and phylogeography of *Echinococcus granulosus* genotypes G6-G10 based on complete mitochondrial genomes and six nuclear loci

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Introduction: Cystic echinococcosis (CE) is a zoonotic disease for which the etiological agent is the larval stage of the species complex of *Echinococcus granulosus*. It is well established that this species complex displays wide genetic diversity with 8 recognised genotypes. While the species status of some genotypes seems to be resolved, the taxonomy of G6-G8 and G10 has remained controversial. Previous studies have mainly used the mitochondrial genome (mtDNA) in taxonomic studies. However, for species delimitation it is crucial to include data from nuclear loci as mtDNA only reveals the evolutionary history of the maternal lineage. Moreover, the distinction between G6 and G7 has been ambiguous.
Objectives: One of the main aims of this study was to include for the first time all four genotypes in a nuclear and mtDNA genome analysis in order to help elucidate the species status of G6-G8 and G10. Additionally, we explored for the first time the genetic diversity and phylogeography of G6 and G7 based on complete mtDNA.
Material and methods: We sequenced full mitochondrial genomes for phylogeographic analyses and to correctly identify genotypes, as well as 6 nuclear loci for phylogeny analysis**.**
Results and conclusions: The mtDNA analysis revealed 4 distinct mtDNA lineages, while the nuclear data showed only 2 clusters, G6/G7 and G8/G10. Therefore genetic evidence suggests the division of these 4 genotypes into 2 species. Based on phylogeographic and genetic variability data of G6/G7, we concluded that: (i) complete mitogenome data allows for a significantly better phylogenetic and –geographic resolution; (ii) G7 is represented by two major haplogroups G7a and G7b; (iii) animal trade has had an impact on the genetic structure of G6 and G7, making it challenging to detect clear geographic segregation patterns; (iv) we also found one highly divergent sample from Mongolia, which did not clearly cluster into neither G6 nor G7.

ABSTRACTS

Drug resistance

DRE-O-01

Predicting drug resistance evolution

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Drug resistance constitutes one of the most pressing veterinary and public health concerns caused by parasite, pathogen and cancer evolution alike. The current focus on prudent use and drug development is important, but studying the evolutionary processes leading to drug resistance is mostly neglected. Here I will discuss how we can harness a pharmacological framework to predict the evolution of drug resistance. I will illustrate this approach by comparing resistance evolution of bacteria against antimicrobial peptides with antibiotics, but the same framework is applicable to other drugs such as antihelmintic or anti-malarial drugs. The theoretical analysis of resistance evolution finds that pharmacodynamic differences all combine to produce a much lower probability that resistance will evolve against antimicrobial peptides. This conceptual framework is widely applicable and can help avoid resistance evolution if implemented in treatment regimens or the rational choice of new drug candidates. It hence provides a tool for sustainable drug use.

DRE-O-02

Macrocyclic lactones: activation of a new subtype of glutamate-gated chloride channels in *Parascaris* sp.

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Parascaris sp. is the largest nematode parasite of equids and is responsible for colic and death in foals through intestinal obstruction. Parasite control largely relies on the use of anthelmintics including ivermectin and moxidectin that act on glutamate-gated chloride channels (GluCls). However, *Parascaris* sp. resistance to macrocyclic lactones is a growing issue over the recent years throughout the world. Moreover, the GluCls of *Parascaris* sp. targeted by macrocyclic lactones are unknown. Here we identified and cloned the cDNAs of *Peq-avr-14b* and *Peq-glc-2* encoding two GluCl subunits and investigated them at the functional level.
The subunits Peq-AVR-14b and Peq-GLC-2 were expressed singly and in combination in *Xenopus laevis* oocytes to explore their function and pharmacology. Two-electrode voltage clamp recordings showed that each subunit could not form an homomeric channel. Interestingly, we have found that the co-expression of Peq-AVR-14b and Peq-GLC-2 led to a novel functional GluCl subtype. Moreover, we demonstrated that moxidectin and ivermectin were more potent agonists than glutamate. We also detected a SNP in the *Peq-avr-14b* gene from ivermectin-resistant worms that may encode a truncated form of the subunit. Its functional relevance in ivermectin resistance is in progress.
Here we report the functional characterisation of the first moxidectin/ivermectin-sensitive GluCl of *Parascaris* sp., thus opening the way for better understanding the mode of action of macrocyclic lactones and resistance mechanisms in ascarids.

DRE-O-03

Cytochrome P450 expression in benzimidazole susceptible- and resistant isolates of *Haemonchus contortus* following exposure to thiabendazole

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Introduction: Decades of uncritical benzimidazole (BZ) use has selected many resistant parasitic nematode populations in sheep. Polymorphisms in the isotype 1 β-tubulin gene can cause resistance but isolates carrying 100% of a resistance-associated allele can display differences in their resistance levels. Cytochrome P450 monooxygenases (CYPs) are linked to drug resistance in mammals and arthropods and were suggested to also contribute to BZ resistance in nematodes.
Objectives: The study aimed to compare basal and thiabendazole (TBZ)-inducible mRNA expression levels between *Haemonchus contortus* isolates with different, well characterized BZ resistance status.
Materials & Methods: Using qRT-PCRs, seven CYPs were investigated regarding constitutive and TBZ-inducible expression after 3 h and 6 h exposure of in vitro generated fourth stage larvae of two susceptible and three resistant *Haemonchus* isolates. Selected CYPs are orthologues of the BZ/xenobiotic-inducible CYP35 and the xenobiotic-inducible CYP31A families in *Caenorhabditis elegans*, as well as the CYP13A family. Resistance status of all isolates was determined by egg hatch assays (EHA) and pyrosequencing.
Results: The *H. contortus* isolates showed a wide range in phenotypic resistance to TBZ in the EHA. The frequency of the F200Y mutation correlated with phenotypic resistance but the WR isolate showed extremely high resistance levels. No significant changes were observed in the expression of all investigated CYPs following exposure to TBZ. Comparison of basal transcript levels revealed a significantly higher expression of CYP HCOI100383400 in the highly resistant WR isolate when compared to both susceptible HcH (2.4-fold) and CAVR (2.7-fold) isolates and the intermediately resistant IRE (3.7-fold) isolate.
Conclusion: In a multi-genic context, the constitutive overexpression of CYP HCOI100383400 might lead to additive or synergistic effects and thus contribute to a highly BZ-resistant phenotype.

DRE-O-04

Genetic basis of benzimidazole resistance in *Caenorhabditis elegans* wild isolates

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Benzimidazoles (BZ) are essential components of the limited chemotherapeutic arsenal available to control the global burden of parasitic nematodes. In veterinary medicine, extensive treatment on livestock has generated geographically widespread BZ resistance and raised the concerns that selective pressure of mass drug administration may lead to reduced cure rates in human populations as well. Although single nucleotide mutations in beta-tubulin genes were identified to be major determinants of BZ resistance in parasitic nematodes, there is increasing evidence that BZ resistance is a polygenetic trait. To improve treatment strategies, a more detailed understanding of the mechanisms leading to BZ resistance is of high importance.

Since population genetics approaches are strongly limited in helminth parasites we performed genome wide association (GWA) studies on a wild isolates panel of the free-living model nematode *C. elegans*. We evaluated the variation in BZ responses of 240 genetically divergent strains using the COPAS BIOSORT platform, which allows high-throughput measurement of multiple fitness traits including brood size and growth rate. Subsequent association mapping was performed with a genome-wide set of 13,540 single nucleotide variants as genomic markers leading to the identification of several quantitative trait loci (QTL) that contribute to resistance. Beside QTL that were found to be common among different BZ, some QTL appear to be drug-specific.
Additionally, we did an in-depth analysis on *ben-1*, a beta-tubulin gene coding for the major drug target in *C. elegans*. This approach revealed unexpected genetic variation among strains at this locus including coding variants, insertions, and deletions that could be linked to resistance. These findings provide further evidence for the complexity of BZ resistance and have the potential to expand our knowledge of the genetic mechanisms that contribute to anthelmintic drug response in nematodes.

DRE-O-05

Occurrence of insecticide resistance in stable flies (*Stomoxys calcitrans*) on dairy farms in the federal state of Brandenburg, Germany

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Stable flies are an ongoing problem in animal husbandry worldwide. Since they can have a considerable negative impact on animal well-being, health and productivity, the use of insecticides represents the mainstay for their control. This study aimed at assessing the occurrence of insecticide resistance in *S. calcitrans* on dairy farms in Brandenburg, Germany, and at proposing strategies for on-farm pest control thereby minimizing the use of insecticides.
Based on a questionnaire analysis, the susceptibility of flies from 40 dairy farms to a deltamethrin-impregnated fabric was tested using the FlyBox® method. For confirmatory purposes, *S. calcitrans* populations from 10 farms were reared in the laboratory and the emerging offspring was tested under controlled conditions against both, the synthetic pyrethroid deltamethrin and the phosphoric acid azamethiphos, by topical application of the calculated discriminating dose (LD95) and multiples of it. Furthermore, the larvicidal effectiveness of the insect growth regulators cyromazine and pyriproxyfen was evaluated at different concentrations based on the manufacturers" recommended doses.
The FlyBox® method indicated 100 % resistance against deltamethrin. In the laboratory, when comparing to the LD95, 24 hours following topical application of deltamethrin and azamethiphos, mortalities below 90 % were encountered for the 10 established lab strains. This led, hence, to the conclusion that these strains were resistant to the tested insecticides. Likewise, exposure to the larvicides cyromazine and pyriproxyfen at their recommended concentrations demonstrated 100 % inhibition of the moulting process of the populations tested.
Continuous or non-strategic use of insecticides may lead to the development of insecticide resistance. World-wide, further in-depth studies in different livestock production systems as well as constant vigilance are a prerequisite for minimizing the risk of insecticide resistance development on a global scale.

DRE -O-06

More than immune evasion – a variant surface glycoprotein causes *in vitro* suramin resistance in *Trypanosoma brucei*

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Suramin is the drug of choice to treat the first stage of the acute form of sleeping sickness, caused by *T. brucei rhodesiense*. Despite its use for a century, knowledge about the drug is still limited. Investigating drug resistance mechanisms can help to identify the target, transport and mode of action of a drug. For suramin, we observed the emergence of a high resistance in a *T. b. rhodesiense* strain (STIB900) after exposure to the drug for only few days. Here we investigate the genetic and biochemical mechanisms behind this phenomenon.

A fresh STIB900 clone was exposed to suramin *in vitro* and four independently selected, resistant derivatives were generated. They were phenotypically characterized and mRNA sequencing was carried out to find differentially expressed genes between suramin sensitive and resistant cells. The identified candidate gene was validated by reverse genetic *in situ* gene replacement. And the effect on endocytosis of different substrates was investigated by FACS and fluorescence microscopy.

Suramin resistant derivatives were obtained after 6 days of selection and showed a resistance factor around 100-fold compared to the sensitive parent clone. They were cross-resistant to trypan blue and had a mild growth defect. Gene expression analysis revealed a switch to the same *variant surface glycoprotein* (*VSG*), termed VSG^{Sur}, in all the resistant derivatives (Fig 1). No other genes were differentially expressed between resistant and sensitive cells. We then introduced VSG^{900}, which was expressed in the sensitive parent clone, into the active expression site of one resistant derivative, and thereby replaced VSG^{Sur} (Fig 2). Through this manipulation, the cells completely lost their resistance. Complementary, the introduction of VSG^{Sur} into the active expression site of *T. b. brucei* 2T1 cells led to suramin resistance. Upon expression of VSG^{Sur}, uptake of trypan blue was reduced, and uptake of low density lipoprotein and transferrin were highly reduced.

We identified a previously unknown *VSG* (VSG^{Sur}), which causes a strong suramin resistance. The expression of VSG^{Sur} not only confers resistance, but also alters the uptake of a number of substrates including nutrients, thus supposedly has a major impact on the biology of the cells.

Figure 1

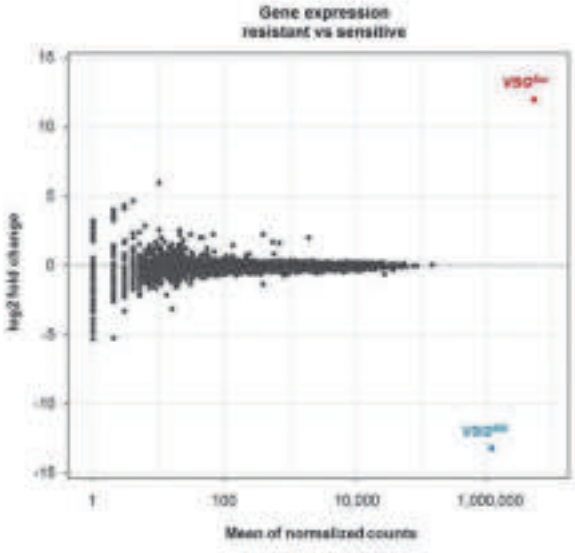
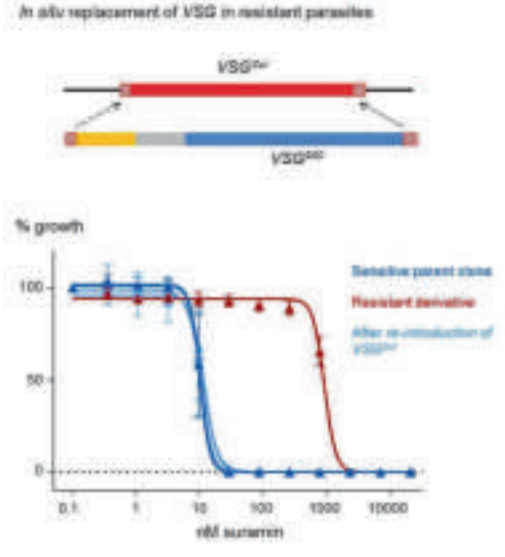


Figure 2



DRE-O-07

Phenotypic characterization of a nitro drug resistant *Giardia lamblia* strain

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Question: Metronidazole and other nitro compounds have been used since five decades as a therapy of choice against giardiasis. As a consequence, resistance formation occurs more and more frequently. Model systems allowing studies on biochemical aspects of resistance formation to nitro drugs are, however, scarce since resistant strains are often unstable in culture. In order to fill this gap, we have generated a stable metronidazole- and nitazoxanide-resistant *Giardia lamblia* WBC6 clone, the strain C4.
Methods: Previous studies have revealed marked differences in the transcriptomes of both strains [1-3]. Here, we present more recent results comparing trophozoites of both strains with respect to their ultrastructure, whole cell activities such as oxygen consumption and resazurin reduction assays, proteomics including key enzyme activities, and several key parameters of oxidative stress such as NAD(P)/NAD(P)H ratios and FAD contents.
Results: Nitro-compound resistant C4 trophozoites have lower nitroreductase activities, lower oxygen consumption and resazurin reduction rates, lower FAD contents and a higher NADH/NAD-ratio than wildtype trophozoites.
Conclusions: The present results suggest that resistance formation against nitro compounds is correlated with metabolic adaptations resulting in a reduction of the electron transfer rate to FAD-dependent oxidoreductases thereby avoiding nitrosative and oxidative stress.
References:
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Parasite ecology

ECO-O-01

The role of parasites in ecology and evolution

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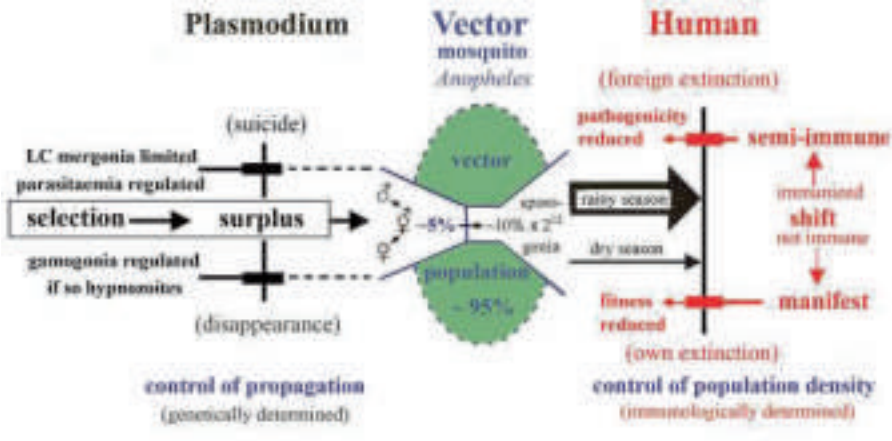
Parasitologic partnerships persist either by *opposing* survival strategy: death or immunity or by *simultaneous* survival strategy, parasite and host propagate synchronously (Wenk & Renz 2012). The endemic *Plasmodium spec.* survive by *selfcontrol* of multiplication combined with *limitation* and *regulation* of parasitaemia. Pathogenicity/lethality is *not relevant.* The survival in the mosquito is achieved by a high-loss *obstacle run*. The particular conditions guarantee the further development, enable fertilisation and new genetic combinations. Only few ookinetes penetrate the peritrophic membrane and enter an intestinal epithelial cell. The sporogony compensates the losses suffered and anticipates the following ones in the haemocoel. In hyper-endemic regions repeated infections reduce the host"s fitness in general. The symptomatology shifts between semi-immunity supported by superinoculation and manifest malaria *without* superinoculation which is obviously regulated by the

frequency of infective mosquito bites. In increasing human age, the losses of *Plasmodium* by the *age drift* caused by the natural fading of the hosts and their spontaneous healing will be compensated by an intensifying of the transmission during the rainy season: the generally decreasing *prevalence* increases temporarily, whereas the *density* of the sexual stages increases. Any parasite propagates exclusively combined with its transmission, the latter of which correlates positively with the contact rate of the host, i.e. its population density. But the parasite diminishes with increasing losses by lethality and/or reduction of host fitness. Thus, feedback limits the host population density and parasite prevalence. Parasitic partnerships correspond to an *insurance contract*: The tolerable costs of a contract (pathology and lethality) are the *premium*, the selective infectivity and susceptibility are the *content* of the contract, and the compatibility is its *closure*. A fundamental change of living conditions is the *insurance case*. Parasitism supports the survival of *both* partners, even when living conditions change. Being optional, they are noticed as a sickness. Missing fossils their selective benefit for evolution has been overlooked hitherto. - Wenk P, Renz A (2012) Historical Biology 25:1-9 – id. (2014) Nat. Rundsch. 67: 276-289.

Fig.: **Symbiontic insurance model of *Plasmodium***.

Left: Multiplication and regulation of parasitaemia (above). Regulated gamogonia (below). **Right:** Immune tolerance shifts between semi-immunity and symptomatical manifest malaria. Foreign eradication (above), excessive host population increase and exhaustion of resources prevented (below). **Middle:** Minimal, involvement of the vector (5%) and its small effectivity (10%) identify it as compatible. Parasite"s loss rate compensated by sporogonia (x 212). Seasonal turnover of the unlimited wild-living vector population shuffles the individual genomes of the *Anopheles* attacking humans (~5% plus ~95%) and prevents the selection of a genetically determined resistance (Wenk und Renz 2014).

Figure 1



ECO-O-02
Factors influencing intestinal parasite infection intensity and parasite community in juvenile spotted hyenas in the Serengeti National Park

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Knowledge is limited on factors determining individual differences in mammal populations in intestinal parasite infection intensity and the composition of intestinal parasite communities. We aimed to investigate factors determining this heterogeneity in juvenile spotted hyena in the Serengeti National Park. Fresh faeces were immediately collected after deposition from known individuals. Conventional coprological methods were used to determine the total burden of parasite egg/oocyte per gm faeces. We hypothesised that infection intensity depended on the parasite transmission mode and the host and parasite environment. We expected infection intensity to decline as age increased because immunocompetence increases and allostatic load declines with age in juvenile hyenas. In this social carnivore, maternal rank determines juvenile nutritional status hence infection intensity was expected to increase as maternal rank declined. *Ancylostoma* is an energetically costly parasite hence the number of concurrent parasite taxa should increase with *Ancylostoma* infection intensity. Finally, monoxenous parasites (*Ancylostoma* and Coccidia) were expected to increase as clan size increased, whereas the indirect transmission route of Diphyllobothriidae through ingestion of intermediate host was not. We found the population harboured seven parasite taxa. Three taxa were the most prevalent, and of these, Coccidia and Ancylostoma egg/oocyte burden was considered a reasonable proxy measure of infection intensity, whereas Diphyllobothriidae egg burden might represent worm fecundity and/or infection intensity. We applied separate models to examine infection intensity for Coccidia, *Ancylostoma* and Diphyllobothriidae. Our results revealed that *Ancylostoma* and Coccidia burden decreased with age. Contrary to expectation, clan size had a negative and no significant effect for *Ancylostoma* and Coccidia burdens, respectively, but increased with Diphyllobothriidae. Furthermore, individuals with low ranking mothers were significantly more likely to have higher egg load of Diphyllobothriidae. Our study identifies some key host factors but also reveal that further research is required to provide more comprehensive answers.

ECO-O-03
A glimpse of the red queen – Long-term experimental co-evolution in a vertebrate-tapeworm system

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One of the strongest selective pressures parasites exert on their hosts (and the following generations) is the impact on its reproductive success. The selective pressure imposed on host reproduction might however vary greatly even between populations of parasites belonging to the same species, depending on their exploitation intensities. In order to prove this general assumption we performed a long-term co-evolution experiment to investigate the impact of the tapeworm parasite

(*Schistocephalus solidus)* on the lifetime reproductive success of its specific host (*Gasterosteus aculeatus)*. To test for effects caused by intrinsic differences in parasite virulence, we included two parasite populations with contrasting, strong differences regarding their exploitation intensity. We replicated the natural life cycles of both species in a controlled laboratory setting, while allowing for responses of host and parasite populations to the selective forces acting on them over multiple generations. This approach allowed for reciprocal multi-generational adaptation on the hosts and parasites side. In my talk I will present data from the first four years of a still ongoing long-term co-evolution experiment and give a perspective on the years to come. Preliminary results showed surprisingly fast adaptive processes with clear indications of selection from standing genetic variation. These processes have thus far resulted in successful adaption of a prior naïve host, displaying a diminishing negative effect on its reproductive success. The generations to come will show how this interaction will play out in the future. We hope the expected counter adaptive processes will give us a glimpse of the red queen in action.

ECO-O-04
Cryptic species and unexpected intermediate host specificity in the acanthocephalan *Polymorphus minutus*

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Polymorphus minutus is a bird-infecting acanthocephalan and was until now the only described species of this genus in central Europe. This parasite is a commonly used model organism for ecotoxicology and behavioural manipulation. Previous data suggests that *P. minutus* comprises different lineages or even cryptic species using different intermediate hosts. Our aim was to test the genetic diversity of *Polymorphus* c.f. *minutus* depending on locality and intermediate host species, to test if *P. minutus* can be considered a cryptic species, and if preferable infection of certain intermediate hosts occurs. We sampled amphipod intermediate hosts infected with *Polymorphus* cf. *minutus* cystacanths originating from 27 sites in Germany and France. Parasites and hosts were identified using integrated datasets (COI and/or morphology for hosts and COI + ITS1-5.8S-ITS2 for parasites). Mitochondrial and nuclear genetic data strongly supported the existence of three cryptic species in *Polymorphus* cf. *minutus* (type 1-3). The three types revealed a high degree of intermediate host specificity: *Polymorphus* type 1 was only encountered in *Gammarus fossarum* type B, *Polymorphus* type 2 in *Echinogammarus* sp. and *Echinogammarus berilloni*, and *Polymorphus* type 3 in *Gammarus pulex* and *Gammarus roeselii*. These results point to a so far neglected cryptic diversity of the genus *Polymorphus* in Central Europe. Furthermore, *Polymorphus* type 2 is most likely a non-native parasite in Germany that co-invaded with *E. berilloni* from the Mediterranean area. Potentially, type 3 originated from South-East Europe and migrated to Germany by *G. roeselii*, where it captured *G. pulex* as intermediate host. Therefore, our findings can be seen in the context of ecological globalization in terms of the anthropogenic displacement of intermediate hosts and its impact on the dispersal of parasite species. Moreover, our data clearly point to a taxonomic revision of *Polymorphus* c.f. *minutus*.

ECO-O-05
Anisakis infection in fish: an eco-parasitological study in different fishing grounds of the Adriatic Sea

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Question: *Anisakis* parasites are found in a large variety of marine hosts. The aim was to map and define the "*Anisakis* risk" in anchovies and sardines caught in the Adriatic Sea, linking the results with environmental and ecological factors.
Methods: 2054 fish were sampled from south-central fishing grounds of the Adriatic sea. The results were compared with those collected from research in the northern area in order to highlight an influence for fish populations toward parasitic infection.
Results: The results showed prevalences for *Anisakis* sp. larvae equal to 8.1% in anchovies and 1.9% in sardines for the central-southern areas. As for the data available for the northern Adriatic Sea, prevalence values found were always below 1%.
Conclusion: Data comparison emphasized that *Anisakis* is more widely distributed in the mid-southern Adriatic Sea than in the northern part since more parasites were detected when moving southward. The differences were linked with the ecology of the sea, that divides it into two ecosystems. The northern portion is a coastal area for its shallow waters and the Italian rivers that provide eutrophic freshwater. These characteristics do not allow a wide abundance of *Anisakis* hosts, because they need deeply and salty waters. In contrast, the central-southern portion is considered as an oceanic ecosystem characterized by higher depth and salinity, as well as oligotrophic waters due to reduced nutrient loads from rivers, allowing a great distribution for the hosts. In conclusion, the ecology of the Adriatic Sea influences strongly the presence of Anisakis hosts, leading to significant differences in the distribution of larval stages in fish population.
Reference: Costantini E. (2015). Anisakiasi ittica: studio ecoparassitologico su specie ittiche provenienti da diversi areali del Mar Adriatico. Thesis, Master's degree in Marine biology, University of Padua (Italy), supervisors: Prof. Quaglio Francesco and Fioravanti Marialetizia

ECO-O-06
Selection of an entomopathogenic fungus infective for ticks based on biotechnological criteria and virulence
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Ticks are vectors for a multitude of pathogens, causing e.g. Lyme disease and tick-borne encephalitis in many parts of the Northern hemisphere. In Germany 8-10 million people suffer from tick bites every year whereby most bites are caused by *Ixodes ricinus*. At present, there is no individual control measure against ticks available. The overall aim of this project is the development of a novel biological control agent against ticks based on an innovative attract-and-kill strategy.
The basis of the control agent is the attractive effect of carbon dioxide (CO_2) combined with tick-specific attractants and substances causing aggregation in ticks (e.g. aggregation pheromones), slowly

released into the vicinity by a tailored biopolymer formulation. The attractive bead formulation is combined with a kill component, an entomopathogenic fungus (*Metarhizium* spp.), isolated from indigenous dead ticks in Germany. The fungus grows out of the beads and infects the attracted tick. As the ticks are attracted by the beads, a wide-spread and costly application can be avoided.
Aiming at the development of an effective formulation against ticks, three promising *Metarhizium* spp. strains have been studied with regard to biotechnological critera as well as virulence against *I. ricinus* nymphs. A new strain of *M. flavoviride* showed the highest virulence (>65% dead nymphs after 11 days) combined with the broadest pH tolerance between pH 3-9 but the lowest growth rate in liquid culture. In comparison, *M. brunneum* was shown to be less virulent but on the other hand revealed a higher growth rate, both out of beads and in liquid culture. Current experiments deal with drying stability of encapsulated blastospores and sporulation on the bead"s surface for all three *Metarhizium* strains.
This work will pave the way for novel tick control strategies based on an attract-and-kill approach.
Results: Using *Opisthorchis felineus* RNA-seq data, the expression levels of all the genes encoding known components of the system of xenobiotic metabolism and transport were studied on adult and metacercariae stages. As a next step, comparative interspecies analysis was performed using the RNA-seq data from *O. felineus, O. viverrini* and *C. sinensis,* and differentially expressed 'orthologous' coding sequences for detoxification genes were analyzed. The expression of most genes between these three opisthorchiid species was at the similar level independently of the sources of RNA-seq data. As one of the promising molecular targets, the unique parasitic hemoprotein cytochrome P450 (CYP) was tested. Recombinant his-tagged CYP protein was obtained and its binding constants with azole inhibitors were determined. To validate lead compounds for inhibitory effect we studied the action of them *in vitro*, and we found that some of CYP inhibitors have killing effects on *O. felineus* worms in the micromolar range.
Conclusion: Here we present for the first time the results of comparative transcriptomics of Opisthorchiidae liver flukes. This report also presents the first biochemical and catalytic study of the CYP enzyme of any of the parasitic flatworms. We have been characterized its biophysical properties and found its micromolar inhibitors, that have killing effects on worms.

Epidemiology and emerging infections

EPI-O-01
Spread of human dirofilariasis in Europe

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Introduction and objectives: Dirofilariasis is considered an emerging infectious zoonosis in Europe. Humans are aberrant hosts, but infections can cause subcutaneous nodules and even cases of meningoencephalitis were observed. Recent reports indicated a noticeable spread of the disease from the Mediterranean to Eastern and Central Europe.
Materials & Methods: In order to understand the spread of the disease over the past decades, the correlation between the length of the potential annual transmission periods and reports of human cases were analysed to determine the threshold of days differentiating between areas with and without risk of *Dirofilaria* transmission to humans.
Results: Due to rising temperatures in Europe, the annual periods of time suitable for *Dirofilaria* transmission became longer for many regions in Eastern and Central Europe resulting in a higher risk

of human infections, which clearly corresponds to the increased number of published human case reports.
Conclusion: The spread of dirofilariasis in Europe is directly linked to an increase of the annual time windows allowing extrinsic incubation. While the spatial extent of the area under risk did not change significantly until the end of the 20th century, the disease showed a tremendous spread within the past two decades. Due to the clear temperature dependence of the parasite, rising temperatures in course of the climate change will probably result in a further spread of human dirofilariasis in Europe.

EPI-O-02
Molecular identification of tick-borne pathogens infecting cattle in Asia and Africa reveals emerging *Anaplasma*, *Ehrlichia* and *Babesia* species

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Tick-borne diseases affect the health and productive performance of cattle, particularly in the tropics. To elucidate the epidemiology of tick-borne pathogens (TBPs) in cattle, five cross-sectional studies were performed in Bangladesh, Ethiopia, Mongolia, Pakistan and South Sudan between 2011 and 2016. In these countries, blood samples and/or ticks were collected from clinically healthy cattle. DNA was subsequently extracted from these samples and screened by polymerase chain reaction (PCR) and a Reverse Line Blot (RLB) hybridization assay for the presence of *Anaplasma*, *Ehrlichia*, *Rickettsia*, *Babesia* and *Theileria* species.
The RLB results demonstrated the presence of DNA from multiple TBPs in these samples, with frequent occurrence of co-infections. In addition to well-characterized TBPs, eight previously uncharacterized *Anaplasma*, *Ehrlichia* and *Babesia* species were also identified and phylogenetically analysed. The pathogenicity and vectors of these species remain to be determined.
These findings highlight the complex patterns of TBP co-infections in cattle and demonstrate the advantage of the RLB assay in the discovery of novel tick-borne pathogens.

EPI-O-03
Prevalence of metastrongyloid lungworm larvae in Colombian giant African snails (*Achatina fulica*)

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Introduction: Human infections with metastrongyloid *Angiostrongylus* spp. may cause severe gastrointestinal or neurological diseases. To date, *A. costaricensis* is endemic in Colombian wildlife

animals whilst *A. cantonensis* has never been reported in the Colombian territory. In addition, canine and feline lungworms (*A. vasorum, Crenosoma vulpis, Aelurostrongylus abstrusus*) were scarcely reported in South American wildlife animals and represent neglected parasites in Colombia. Research on these lungworm infections in Colombia is therefore required to gain actual information on the respective prevalence. Furthermore, given that six endangered wild felid species are present in Colombia, it is of great interest to determine actual data on the parasitoses which may threaten these populations.
Objective: Since all of these nematodes include snails/slugs as intermediate hosts, we here analysed Colombian giant snails (*Achatina fulica*) from different regions of Colombia for the presence of *A. vasorum*, *C. vulpis*, *T. brevior*, *A. costaricensis*, *A. cantonensis* and *A. abstrusus* infections.
Material and Methods: In total, 609 *A. fulica* were collected from the following regions of Antioquia and Putumayo: Tulúa, Andés, Ciudad Bolívar, Cañasgordas (all urban zones) and Puerto Leguízamo (Amazonian region). The snails were cryo-euthanized, submitted to artificial digestion via pepsin/HCl treatments and examined microscopically for the presence of metastrongyloid larvae. Larvae-positive samples were confirmed via species-specific PCR followed by sequencing.
Results: Whilst no infections with *A. costaricenis* and *A. cantonensis* were detected via molecular biological techniques, *A. fulica* proved positive for *A. abstrusus* (9.4 %), *A. vasorum* (3.9 %), *Crenosoma vulpis* (1.1 %) and *Troglostrongylus brevior (*1.3 %) larvae.
Conclusion: The current study presents the first report on *A. abstrusus, T. brevior, C. vulpis* and *A. vasorum* infections in Colombian intermediate hosts.

EPI-O-04
Epidemiology of *Acanthamoeba* infections

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Acanthamoeba spp. are the causative agents of a painful inflammation of the cornea, the so-called *Acanthamoeba* keratitis (AK), on one hand and on the other hand of several disseminating infections potentially resulting in granulomatous amoebic encephalitis (GAE) in the immunocompromised host. The first cases of AK were reported in the early seventies and in the mid-eighties an association between AK and contact lens wear was discovered. Today, acanthamoebae, besides pseudomonads and staphylococci, are regarded as the most important causative agents of keratitis in contact lens wearers. AK often shows a severe progression, which is due to a lack of awareness but also to the lack of specific treatment. The dormant cysts pose a particular problem, often residing within the tissue and leading to reinfection after termination of treatment. In industrialised countries the annual incidence of AK lies between 0.1-1 cases per 100,000 inhabitants, with a marked regional variation depending on contact lens wear habits and mode of water supply. In some areas there is a clear peak of AK cases during the summer months. In Austria, the annual incidence of AK currently lies around 0.16 cases per 100,000 inhabitants, with >90% of cases occurring in contact lens wearers. In contrast to the early years, today, males and females are equally represented and the most affected group are the 21-30-year-olds. The figures for GAE are much lower, less than 500 cases of GAE have been published worldwide since its discovery. At the Austrian reference institution, *Acanthamoeba* diagnostics was established in 1993. Since then, 180 cases of AK and 6 cases of GAE were diagnosed. The most common genotype in human *Acanthamoeba* infections is T4, which also is most abundant genotype. Other genotypes rather frequently isolated are T3 and T11 from AK patients, and T1, T10 and T12 from GAE.

EPI-O-05
Leishmaniasis in Uzbekistan – an epidemiological update

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Background: In Uzbekistan three forms of leishmaniasis are registered: visceral (VL), zoonotic cutaneous (ZCL) and anthroponotic cutaneous leishmaniasis (ACL). Historically Uzbekistan was the republic with the highest leishmaniasis prevalence in the former USSR. In the first half of the 20th century > 6000 VL cases occurred, however due to intensive control measures leishmaniasis was nearly eradicated by the end of the 1960s. VL is re-emerging since the 1990ies. In the last two decades the number of leishmaniasis cases is continiously increasing. The objective of this study is to present an epidemiological update and implementation of modern molecular diagnostics and ecological niche modeling for risk assessment of leishmaniasis in Uzbekistan.
Methods: Between 2014-2016 103 clinical samples (12 VL and 91 CL cases) from six different endemic regions of CL and VL (Samarkand, Jizzakh, Navoi, Kashkadarya, Surkhandarya, Fergana) were studied. In addition, reservoir studies were carried out in the Kashkardarya region and entomological surveys in the endemic regions Jizzakh, Surkhandarya, Samarkand, Kashkardarya. Molecular identification of the causative agents at species level was carried out followed by pilot multilocus microsatellite typing (MLMT) to reveal the diversity of circulating genotypes at strain level. Ecological niche modeling was performed based on registered cases and entomological data and spatiotemporal distribution models were calculated for risk assessment.
Results: 2013-2015 a total of 916 CL cases were registered in 7 regions, whereas the number of VL cases was 52 in 4 regions. ITS1-RFLP of the collected clinical samples identified the causative agents of CL as *L. major* and *L. tropica*, and of VL as *L. infantum*. MLMT placed *L. infantum* from Uzbekistan in a cluster close to *L. infantum* MON-1 from Tajikistan, Southern Caucasus and Turkey in a combined data set of 520 strains from 19 countries, and *L. tropica* close to a subpopulation of *L. tropica* from Turkey and the Middle East in a combined data set of 269 strains from 12 countries. Spatiotemporal projection based on climatic and environmental conditions identified the main regions at risk in the presence and future.
Conclusions: The present study provides an update of the prevalence of leishmaniasis in Uzbekistan. Moreover, molecular diagnostics was established for the first time in Uzbekistan. The etiology of leishmaniasis was defined in the studied regions and for the first time causative agents of CL were typed by molecular methods at species and strain level. Important conclusions about spatial and temporal dynamics of the disease could be provided by inferring species distribution models allowing development of appropriate control measures.

EPI-O-06

Emerging foci of visceral leishmaniasis in Armenia – molecular epidemiology and pilot risk assessment by ecological niche modeling

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Background: Visceral Leishmaniasis (VL) is re-emerging in Armenia. From 1926 till 1969 919 cases were reported in 16 districts and dogs were identified as reservoir. After a control program in 1954-1969 VL disappeared until 1999 when again a single case emerged. Until 2016 >81 cases were recorded in different parts of Armenia. Entomological studies in active foci identified *Ph. balcanicus* and *Ph. kandelaki* as main vectors. Objectives of this study were (i) to determine for the first time the genetic diversity and population structure of *L. infantum* in Armenia and to compare the genotypes with those from endemic regions worldwide to draw conclusions about the mode of spread and to enable epidemiological monitoring of VL in Armenia; (ii) to predict the spread of VL and the vectors in time and space based on ecological niche modeling to provide control strategies.
Methods: 20 human samples from different parts of Armenia previously identified by ITS-1 RFLP as *L. infantum* were studied by Multilocus Microsatellite Typing (MLMT). These data were combined for comparison with the set of all previously typed *L. infantum* strains from the main endemic regions in the World (19 countries, 520 strains). Ecological niche modeling was performed based on registered VL cases and identified sandflies collected in 2009-2015. Both datasets were used for calculation of distribution models for different time periods.
Results: Within the 20 Armenian *L. infantum* strains 19 different genotypes were identified, which clustered into two main populations that were not related to the geographic origin of the cases. The combined analysis revealed the closest relationship to MON-1 subpopulations from Greece, Turkey, Cyprus, Israel, Uzbekistan. The ecological niche models identified regions at the borders to the neighboring countries Georgia, Turkey, Iran, Azerbaijan as most suitable for the vectors and with the highest risk for VL.
Conclusion: This study identified for the first time genotypes and their diversity of *L. infantum* circulating in Armenia assigning them to specific geographic MON-1 populations. Based on these results further genotyping studies should be performed with human and animal reservoir samples also from the neighboring countries to understand re-emergence, spread and epidemiology of the disease. An important role for developing control strategies in the whole region of Southern Caucasus will play further modeling based on a comprehensive collection of epidemiological and entomological data in this region.

EPI-O-07
Investigation of causative agent of cat scratch disease in cats and their fleas in Lithuania

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Introduction: *Bartonella* are vector-borne bacteria causing zoonotic diseases. Cats can be infected with several *Bartonella* species. Cat scratch disease is frequent worldwide zoonosis caused by *B. henselae* and *B. clarridgeiae*. Cats are a major reservoir for this two *Bartonella* species. Cat fleas are vector for transsmision of the infection agents among cats. Human can be infected via a scratch or bite of the cat, also bite of the cat flea.
Objectives: The aim of this study was to investigate the presence of *Bartonella* infections in cats and their fleas in Lithuania.
Materials and Methods: Blood samples were collected from 46 cats presented in pet clinics and animal shelter. A total 102 fleas representing two species were collected from cats. *Bartonella* DNA in samples was detected using a nested-PCR of the ITS region. Positive PCR products were selected for DNA sequencing.
Results: *Bartonella* species were detected in 7 (15.2%) out of 46 cats, and 21 (20.6%) out of 102 fleas. *Bartonella* were detected in 16 out of 92 of *Ctenocephalides felis* flea species, and 5 out of 10 *Ct. canis* flea species. The ITS region sequences showed that both, *B. henselae* and *B. clarridgeiae* were detected in cats and fleas. *B. henselae* was more common than *B. clarridgeiae*. Three *B. henselae* strains were identified.
Conclusions: This study is the first report on prevalence and molecular characterization of *B. henselae* and *B. clarridgeiae* in cats and cat fleas in Lithuania

Immunology

IMM-O-01
The outcome of *Entamoeba histolytica*-induced liver damage is influenced by sex-dependent activation of macrophages and monocytes

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Introduction: The amebic liver abscess (ALA) is a severe focal destruction of the liver and is caused by the protozoan parasite *Entamoeba histolytica*. The tissue destruction is mediated by the pro-inflammatory IL-23/IL-17 axis with subsequent CCL2-dependent recruitment of Ly6Chi monocytes and the production of TNFα. Since the incidence of an ALA is higher in male than in female individuals, in human as well as in the murine model, it provides a platform to elucidate the differences between female and male immune responses.

Objectives: We aim to analyze the differences of female and male monocyte activation with regard to differently stimulated macrophages, displaying residential cells.
Materials and Methods: To investigate stimulatory differences between female and male individuals, macrophages were obtained from healthy blood donors and generated out of CD14+ monocytes. Subsequent stimulation with soluble amebic antigens, lipopolysaccharide (LPS) or lipoteichoic acid (LTA) led to a cytokine and chemokine production as determined by a multiplex bead-based immunoassay. The macrophage supernatant was used to stimulate freshly isolated monocytes, which were further characterized via FACS analysis.
Results: We found sex- and stimulus-dependent influences on cytokine secretion of human macrophages and on the surface marker expression of monocytes. Especially the release of the cytokine TNFa was significantly increased in male-derived macrophages following the stimulation with bacterial antigens.
Conclusion: In regard to ALA, we assume that an increased production of a pro-inflammatory cytokine, like TNFα, might prone men towards a TNFα-dependent immunopathology in hepatic amebiasis.

IMM-O-02
Intestinal oncosphere invasion of *Echinococcus multilocularis*: Investigation of resistance mechanisms in a new rat model

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Introduction: Alveolar echinococcosis (AE) is considered a serious chronic parasitic disease in the northern hemisphere, caused by the metacestode of the fox tapeworm *E. multilocularis*. Interestingly, susceptibility and resistance to oncosphere invasion and subsequent AE development widely vary among mammal species. Immunocompetent rats are considered resistant to oral infection, however, if treated with dexamethasone, the barrier mediating resistance is broken, leading to the hypothesis that immune mechanisms are involved in the resistance of rats against oncosphere invasion.
Objectives: The aim of this study was to address the role of innate immune cell populations such as natural killer (NK) cells, macrophages (MΦ) and granulocytes (PMN) in a naturally resistant host following infection with *E. multilocularis* eggs.
Materials and Methods: Wistar rats were depleted of selected immune cell populations by specific antibodies (NK cells and PMN) or clodronate-liposomes (MΦ). Metacestode development in the rat"s livers was analyzed at necropsy 10 weeks p.i. and antibody development against metacestode vesicle fluid (EmVF) was evaluated.
Results: Cell depletion at day of egg inoculation was shown in all individual depleted animals. All control rats were AE and antibody negative at time of necropsy. Whereas NK cell and MΦ depletion had no impact on parasite growth, 9/14 PMN depleted rats showed AE development in the livers, indicating that PMN are key players in preventing oncosphere invasion. Most of AE infected animals revealed antibodies against EmVF.
Discussion/Conclusion: Previous research on the immune response to *E. multilocularis* infection was limited to factors involved against metacestode development and growth in the liver. This new animal model will allow studying the early oncosphere invasion process in a naturally resistant host species. *In*

vivo and *in vitro* experiments are currently ongoing to further characterize the cellular and molecular mechanisms of resistance against *E. multilocularis* infection by PMN.
Reference: Joekel and Deplazes 2017: Optimized dexamethasone immunosuppression enables *Echinococcus multilocularis* liver establishment after oral egg inoculation in a rat model

IMM-O-03
Site-specific effects of IL-33 treatment during helminth infection: increased parasite burden in the tissue and reduced parasite burden in the intestine

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Introduction: *Strongyloides ratti* is a rodent specific parasitic nematode that displays tissue migrating and intestinal life stages. Infective larvae actively penetrate the skin of their host, migrate within 2 days via the skin and lung to the mouth. They are swallowed, reach the intestine and moult to adults that reproduce by day 5. Infected mice terminate the infection in the context of a type II immune response within 4 weeks. Thereby, infection-induced expression of the alarmin IL-33 by alveolar epithelial cells was shown to promote type II response in the lung and was associated with efficient expulsion of *S. venezuelensis* from the intestine (Yasuda *et al.*, 2012 PNAS 9: 3451)
Objective: In this study, we intend to dissect IL-33 mediated effects on the immune response to *Strongyloides* infection in the tissue and the intestine.
Methods: Mice were treated with recombinant IL-33 (rIL-33) intraperitoneal (i.p.) or intranasal (i.n.) and numbers of migrating larvae in tissue and parasitic adults in the intestine were counted. Mucosal mast cell degranulation was quantified by measuring serum concentrations of mouse mast cell protease 1.
Results: *S. ratti* infected mice showed a drastic reduction of parasitic adults in the intestine on day 6 after previous i.n. and i.p. treatment with rIL-33. The reduced parasite burden correlated with increased mast cell activation that is central for expulsion of *S. ratti* from the intestine. Of note, also rIL-33 administration after the tissue migration phase (day 4 and 5) reduced intestinal parasite burden. In contrast, numbers of migrating larvae in skin, lung and head were significantly increased in rIL-33 treated mice indicating that the reduced intestinal parasite burden is not due to an improved immunity in the tissue.
Conclusion: In summary, our data show that IL-33 displays contradictory and site specific effects on tissue migrating and intestinal *S. ratti* parasites. We are currently investigating the underlying mechanism.

IMM-O-04
Dry season *P. falciparum* asymptomatic infections' impact on the host immunity

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Although much is reported on the immune response *Plasmodium falciparum* causes during clinical malaria in the wet season, the impact of silent parasatiaemias on the host"s immunity during the dry season is scarcely studied. During the six-month dry season in Mali, transmission is close to zero but *P. falciparum* (Pf) remains asymptomatically in 30% of the children assuring *de novo* transmission to *Anopheles* mosquitoes in the ensuing wet season.
To address the impact of silent parasitaemias on the host immunity, we compared serological markers of inflammation, *P. falciparum* specific humoral responses, cytokine production and cytotoxic function in Pf+ and Pf- children during the dry season. We further determined if invasion inhibition capacity is different in Pf+ and Pf- children at the end of the dry season, and phagocytic capacity of monocytes from Pf+ and Pf- children at the end of the dry season. Using PBMCs, we also analysed *ex-vivo* T-cells, B-cells, Natural Killer cells and Monocyte populations and their subsets in Pf+ and Pf- children at the end of the dry season and investigated functional markers within these populations.
We observed that serologic markers of inflammation remain unaltered between the beginning and end of the dry season in Pf+ or Pf- children and PfEMP1-specific as the majority of *P. falciparum* humoral responses decrease regardless of the infection status of the host. We observe no difference in invasion inhibition in Pf+ or Pf- children, although depleting antibodies from plasma increases invasion efficiency in all donors and we observe no consistent differences in Pf+ and Pf- children in cellular adaptive immunity during the dry season.
Our data highlights the apparent minimal effect of asymptomatic *P. falciparum* infection on the host immunity during the dry season and suggesting that parasites that are maintained chronically for several months are less able to drive an immune response than recently transmitted parasites.

IMM-O-05
Molecular mechanisms of bovine NET formation induced by *Toxoplasma gondii* tachyzoites

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Introduction*: Toxoplasma gondii* tachyzoites were recently demonstrated as potent inducers of neutrophil extracellular trap (NET) formation in the human system. Via this innate effector mechanism, polymorphonuclear neutrophils (PMN) are capable of entrapping tachyzoites, thereby

immobilizing them and potentially preventing them from host cell invasion. Thus, NETosis may play an important role for the outcome of the disease in the *in vivo* situation.
Objective: The current study analysed *T. gondii*-triggered formation of neutrophil extracellular trap (NET) and ROS production in the bovine PMN system. Here, the role of intracellular Ca++-levels, of selected signalling pathways and of PMN-derived autophagy was analysed.
Material and Methods: *T. gondii* tachyzoites were co-cultured with freshly isolated bovine PMN at ratio of 4:1. NET formation was evaluated by spectrofluorescence and microscopy. Intracellular and extracellular ROS production was estimated using the fluorescent probes DCFH-DA and Amplex red, respectively. For functional inhibition assays, the following inhibitors were used: UO126, (inhibitor of ERK 1/2 pathway), LY294 (inhibitor of PI3K pathway) and 2-APB (Inhibitor of Store-Operated Calcium Entry).
Results: We here show that *T. gondii* tachyzoites significantly trigger NETs in bovine PMN. Overall, tachyzoite-induced NET formation proved dependent on both, intracellular Ca++- concentration and MAPK-related pathway. Neither extracellular nor intracellular ROS was increasingly detected in the current experimental settings. In addition, PMN-derived autophagy did not play a major role in NETosis since the autophagy modulators rapamycin and wortmannin both failed to influence parasite-triggered reactions.
Conclusion: We here confirmed *T. gondii* tachyzoites as potent inducers of NETosis and added some details on molecular mechanisms of parasite-triggered NETs.

IMM-O-06
***Dirofilaria immitis* microfilariae and third stage larvae induce different types of NETs in canine PMN**

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Introduction: *Dirofilaria immitis* causes heartworm disease. Third-stage larvae (L3) are transmitted via culicid mosquitos. Adult nematodes reside in pulmonary arteries and the right heart releasing microfilariae (Mf) into the bloodstream leading to chronic and sometimes fatal disease. So far, early innate immune reactions triggered by *D. immitis* stages in the canine host have scarcely been investigated.
Objectives: In the current study we analysed vital *D. immitis* Mf and L3 stages for their capacity to induce neutrophil extracellular traps (NETs) in canine PMN.
Material & Methods: Canine PMN were exposed to vital or heat-killed *D. immitis* stages and NET formation was analysed microscopically or by measuring extracellular DNA intensities. For co-localisation studies, parasite-triggered NET structures were stained for DNA via Sytox Orange and for histones, neutrophil elastase (NE) or myeloperoxidase (MPO) by specific antibodies. NET inhibition experiments were performed using a NADPH oxidase inhibitor (DPI). For NET resolution, DNase I treatments were used.
Results: SEM analysis revealed Mf and L3 as strong inducers of canine NETosis. Co-localisation of extracellular DNA with granulocytic histones, NE or MPO in parasite-entrapping structures confirmed classical characteristics of NETosis. NETs were induced in a time-dependent but dose-independent (worm/cell ratio) manner by both larval stages and proved independent from parasite viability. Parasite/PMN confrontation promoted significant entrapment but not killing of Mf and L3. Both,

NETosis and larval entrapment was reversed via DNase I but not by DPI treatments. Interestingly, different types of NETs were induced by *D. immitis* stages since Mf merely induced spread and diffuse NETs whilst L3 additionally triggered aggregated NET formation.
Conclusion: Given that *D. immitis* stages might be hampered from adequate migration and development *in vivo*, canine NETs may represent an important effector mechanism acting against *D. immitis* Mf and L3.

IMM-O-07
S100A9 knockout increases inflammatory immune responses upon *L. sigmodontis* L3 larvae and impairs larval migration

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Using the *Litomosoides sigmodontis* mouse model of filariasis, in which infective L3 larvae migrate from the site of infection in the skin via the lymphatics and the pulmonary capillaries to the thoracic cavity where the adult filariae reside, we demonstrated that neutrophils are essential to mediating protective immune responses within the skin against invading L3 larvae and contribute to L3-induced lung pathology. S100A8 and S100A9 are damage-associated proteins that are highly expressed by neutrophils and increasingly found in the lung during the acute phase of *L. sigmodontis* infection. Herein we investigated the impact of S100A9 on *L. sigmodontis* infection using S100A9-/- C57BL/6 mice.
Following natural infection with *L. sigmodontis*, S100A9-/- mice had a significantly reduced worm burden at 12 days post infection (dpi) that was also observed following subcutaneous infection, which circumvents the skin, the first barrier infective L3 larvae have to bypass. This reduced worm burden in S100A9-/- mice correlated with increased frequencies of neutrophils, macrophages and eosinophils as well as increased levels of CXCL1, CXCL2, CXCL5, ENA-78 and elastase in the bronchoalveolar and thoracic cavity lavages. Furthermore, S100A9-/- mice had higher levels of neutrophil activation *in vitro* and *ex vivo* in comparison to wild type mice. Interestingly, upon intravenous injection of L3 larvae, S100A9-/- mice had an increased worm burden, suggesting that the simultaneous entrance of the L3 larvae from the pulmonary capillaries into the thoracic cavity reduces inflammatory responses L3 larvae encounter, overcoming the protective effects observed in S100A9-/- mice. Thus, S100A9 inhibits L3-induced inflammatory responses in the bronchoalveolar lavage and thoracic cavity, reducing chemokine production and granulocyte recruitment as well as neutrophil activation, facilitating larval migration.

IMM-O-08
Filarial cystatin induced immunomodulation in human monocytes and macrophages

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Introduction: Lymphatic filariasis (LF) is an infection caused by *Wuchereria bancrofti, Brugia malayi or Brugia timori,* a debilitating disease with more than 120 million people infected worldwide. The host-parasite interaction in LF often results in a chronic infection associated with a wide clinical spectrum, characterized by functional dysregulation of both the innate and the adaptive immune responses. The microfilarial larval stage was formerly shown to induce human regulatory monocytes and macrophages. *Brugia malayi* microfilaria (mf) induce a regulatory monocyte phenotype with an upregulation of IL-10 and PD-L1 that correlates with asymptomatically infected individuals.

Objective: One filarial molecule known to counteract host immune responses by inducing IL-10 and regulatory macrophages in mice is filarial cystatin. Notably, *Brugia malayi* cystatin (BmCPI-2) is released in greater amounts by *Brugia malayi* microfilaria compared to the adult stages. Thus, here we aim to determine how filarial cystatin of the human pathogenic filaria *Brugia malayi* contributes to immune hyporesponsiveness in human monocytes and macrophages elicited by microfilaria.
Methods: For this purpose, filarial cystatin was depleted from microfilarial lysate (Mf).
Results & Conclusions: Detecting the immunomodulatory potential of cystatin-depleted Mf revealed that IL-10, but not IL-8 and IL-6 induction in monocytes and macrophages is dependent on the presence of cystatin. In addition, the Mf-induced expression of the regulatory surface markers PD-L1 and PD-L2 in human monocytes, but not in macrophages, is dependent on cystatin. While Mf-treated monocytes result in decreased CD4+ T-cell proliferation in a co-culture assay, stimulation of T-cells with human monocytes treated with cystatin-depleted Mf leads to a restoration of CD4+ T-cell proliferation. Moreover, we show that IL-10 expression in monocytes, but not in macrophages, is independent of ERK signalling, suggesting different pathways of IL-10 induction in these cell populations.

IMM-O-09
Targeting *Ascaris* - specific CD4+ T cells and MHC-restricted T cell epitopes of *Ascaris* antigens

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Intestinal parasitic roundworm infections by *Ascaris lumbricoides* affect about 1 billion people worldwide, leading to chronicity, insufficient immunity to reinfections and malnutrition associated with impaired growth as well as physical and intellectual development. In parallel, *Ascaris suum* causes major economic losses in pig production. The absence of a vaccine against *Ascaris* of humans and pigs despite its urgent need is, among others, a consequence of the lack of knowledge regarding

mechanisms of protection and protective antigens. We therefore aimed at the identification of *Ascaris*-specific CD4+ T cells and T cell antigens.
Using CD40L (CD154) expression as an early TCR activation marker of swine CD4+ T cells, we established a method to study frequency and phenotype of *Ascaris suum*-specific T cells during acute and trickle parasite infection. The CD40L response of porcine CD4+ T cells revealed that parasite excretory-secretory (E/S) products rather than the parasite lysate induced a robust T cell response.
To target immunodominant CD4+ T cells epitopes from *Ascaris* E/S antigens, we applied an *in vitro* reconstituted antigen processing system that allowed us to identify MHCII-restricted peptides for two frequent human HLA-DR alleles. Epitopes from only a few E/S proteins were selected by HLA-DRs from the complex E/S antigen mix and they were overlapping only partially between the different HLA-DR alleles. To test the identified epitopes, we generated human *Ascaris* E/S-responsive CD4+ T cells using antigen-specific T cell enrichment (ARTE) and assessed DR specific epitope selection using CD40L activation, cytokine production and MHCII-Tetramer-staining.
Our approach thereby revealed a novel strategy to identify, characterize and test CD4+ T cell epitopes from complex antigenic sources as basis for targeting the host-protective immunity against *Ascaris* parasites.

IMM-O-10
Helminth-induced interference with vaccination efficacy and the role of type 1 regulatory T cells

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Introduction: Helminths are large multicellular parasites that infect approximately one third of the human population. To avoid their elimination, helminths have evolved sophisticated mechanisms to suppress their host´s immune response. Thereby, not only helminth-specific but also non-helminth-specific bystander immune responses such as vaccine responses are suppressed. We have previously shown that infection of mice with the filarial nematode *Litomosoides sigmodontis* inhibits IgG responses to thymus-dependent model antigens and targets rather T cell help than B cells directly.
Objectives: The aim of the study was to analyze the mechanism of helminth-induced interference with bystander immune responses.
Material and Methods: We measured the humoral response after vaccination with a model antigen and the proliferation of adoptively transferred CD4+ T cell receptor transgenic T cells to analyze T cell functions. Mice were either non-infected, helminth-infected or had cleared the infection. Regulatory T cell subtypes were analyzed by flow cytometry.
Results: Analyzing the kinetics of this suppression we found that antibody responses decreased with duration of concurrent helminth infection. Strikingly, mice displayed suppressed Ig responses even if vaccination was performed 16 weeks after natural termination of the helminth infection. Proliferation of adoptively transferred OT-II T cells was suppressed in *L. sigmodontis*-infected mice and mice that had cleared the infection, reiterating suppressed T helper cell expansion in helminth-infected mice. Foxp3+ regulatory T cells increased locally, but not systemically and were dispensable for *L. sigmodontis*-induced suppression of bystander immune responses. By contrast, we observed a systemic expansion of type 1 regulatory T cells in helminth-infected mice and those with a history of a helminth infection.

Conclusion: In summary, we show that the suppressive status, once established is preserved independently of the presence of living helminth parasites and is associated with an expansion of Tr1 cells.

IMM-O-11
Protozoan co-infection leads to a Th1 immune response in helminth-specific T cells

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Co-infections with parasites or pathogens reflect the natural situation in animals and humans in endemic areas. To decipher the immunological effects of a widespread protozoan infection on the anti-helminth immune response, we studied a co-infection with the enteric nematode *Heligmosomoides polygyrus* in mice previously infected with *Toxoplasma gondii*. The protective immune response directed against the nematode is dependent on parasite-specific Th2 responses. In contrast, *Toxoplasma gondii* infection elicits a strong and protective Th1 immune response. Here, we observed that co-infected animals displayed significantly higher worm fecundity although worm burden remained unchanged. Furthermore the Th2 response to *H. polygyrus* in co-infected animals showed a profound reduction of IL-4, IL-5, IL-13, and GATA-3 expressing T cells. Additionally, co-infected animals showed a lack of eosinophilia and reduced expression of the Th2 effector molecule RELM-β in intestinal tissue. However, the Th1 immune response and parasitemia of *T. gondii* was unaffected by the concurrent nematode infection. Neutralization of IL-12 alone and both IL-12 and IFNγ prior to helminth infection did not restore anti-helminth Th2 responses. Further, we investigated the function of pathogen-specific CD4+ T cells during co-infection. Interestingly, *H. polygyrus*-specific restimulation of splenocytes revealed *H. polygyrus*-reactive CD4+ T cells producing a significant amount of IFN-γ in co-infected animals with diminished IL-4 production. Thus, this data suggests that a previous *T. gondii* infection significantly impairs a helminth-specific Th2 immune response and redirects the helminth-specific T cell response to a Th1 response. Future studies will investigate the underlying mechanisms of dendritic cells in their inability to mount a Th2 immune response during a previous and on-going prominent Th1 infection.

IMM-O-12

Dendritic cell-specific OTUB1 is crucial for a strong and effective immune response against *Toxoplasma gondii* infection

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The importance of dendritic cells in counteracting *Toxoplasma gondii* (*T. gondii*) infection has been largely studied in literature and mice deficient of DCs fail to control toxoplasmosis and rapidly succumb to the infection. One of the most important function of DCs in toxoplasmosis is the production of IL-12, which drives NK cells and T cells production of IFN-γ. IL-12 production by DCs is mediated by *T. gondii* profilin (*Tg*PFN), which activates NF-κB pathway via TLR11 an TLR12. *In vitro* studies may suggest that OTUB1 could regulate NF-kB signaling; however, *in vivo* studies on the molecular function of OTUB1 in inflammatory process have not been reported so far.
To study the DC-specific function of OTUB1 in *T. gondii* infection, we generated CD11c-Cre OTUB1fl/fl mice and infected them with *T. gondii.* Compared with OTUB1fl/fl control mice, CD11c-Cre OTUB1fl/fl mice were more susceptible to *T. gondii*, with enhanced mortality due to impaired parasite control. Although the innate control of *T. gondii* was normal in OTUB1-deficient DCs, IL-12 production of OTUB1-deficient DCs was compromised both *in vitro* and *in vivo*. Interestingly, a 4-day administration of IL-12 enabled CD11c-Cre OTUB1fl/fl mice to control *T. gondii* as efficiently as control mice. We also show that the absence of OTUB1 does not affect the motility and the intracellular pathogen control of DCs before and after *T. gondii* infection. Upon TgPFN stimulation, OTUB1-deficient DCs showed reduced nuclear translocation of NF-κB p65, leading to a diminished production of IL-12 by OTUB1-deficient DCs.
Conclusively, DC-specific OTUB1 is required for potent IL-12 production in *T. gondii* infection, essential for parasite control and survival to infection.

IMM-O-13

Identification of novel receptors contributing to LC3-associated phagocytosis in *Leishmania* infection

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We demonstrated that *Leishmania* (*Lm*) disease development depends on the presence of apoptotic promastigotes in the infective inoculum. We found that apoptotic parasites are engulfed and processed in host macrophages by LC3-associated phagocytosis (LAP). At the same time, viable parasites appear to either evade or survive LAP and start to multiply inducing Leishmaniasis. We hypothesize that uptake of apoptotic parasites by specific receptors and processing in LAPosomes influences disease initiation. In this project we aimed on identification of LAP-inducing receptors involved in uptake of apoptotic promastigotes. We further analyzed possible compartment evasion

mechanisms of viable promastigotes. Isolation and label-free mass spectrometry analyses of dying promastigote-containing phagosomes revealed proteins of the Cathepsin-, Rab- and LAMP-families. Interestingly, complement receptor 3 (CR3) and the phagocytic receptor LRP1 were present in high abundance within the compartment. Blocking experiments revealed CR3 to have a significant influence on the uptake of apoptotic parasites in anti-inflammatory primary human macrophages (hMDMs). Ultrastructural analyses by STEM tomography showed that compartments harboring viable promastigotes have tunnel-like structures protruding from the compartment to the extracellular space. Quantification by immunofluorescence-analyses early after infection of hMDMs indicated about 30% of phagosomes to be connected to the outside. Taken together, our data show CR3 to have a significant impact on internalization of apoptotic parasites. Identified tunnel-like structures could serve as possible evasion strategy of viable parasites to avoid degradation and in turn, affect establishment of disease.

IMM-O-14
Balancing Th2 immunity: Th2/1 hybrid cells in parasite infections and atopy

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Introduction: T helper type 2 responses are central to the control of helminth infections, but also associated with atopic disorders. In several experimental models of worm infection as well as in human strongyloidiasis patients we found CD4+ cells co-expressing signature molecules of Th2 and Th1 cells. Whether Th2/1 hybrid differentiation is a way of preventing overt Th2 inflammation in natural infections, associated with inefficient control of parasitic worms and if such cells are underrepresented in atopic disorders is unclear.
Objectives: To see if Th2/1 hybrid differentiation is common in Th2-driving parasite infections, experimental tick infestations were surveyed. Atopic dermatitis (AD) patients were screened to evaluate if Th2/1 hybrid proportions correlate with disease severity.
Results: Mice infested with the tick *Ixodes ricinus* generated Th2/1 cells, albeit at lower numbers compared to helminth infected mice. Strikingly, Th2/1 cell proportions were highest in the blood and spleen of parasite-exposed mice, while sites of infection and draining lymph nodes harboured relatively more Th2 cells. This was mirrored by IL-4+IFN-g+ hybrids isolated from blood of patients suffering from AD: while circulating CD4+ cells expressing only Th2 cytokines as well as IL-13+IL-22+ and IL-13+IL-17+ cells (all associated with severe AD) were highly enriched in skin homing CD4+ effector cells, few Th2/1 hybrids expressed the skin homing receptor CLA. Patients suffering from severe AD had the lowest proportions of Th2/1 hybrid cells. When *in vitro* models of human skin were exposed to Th2 or Th2/1 hybrid cells, hybrids induced lower skin inflammation.
Conclusion: Our data argue for Th2/1 cells being associated with less severe Th2 inflammation. Whether tipping the balance between conventional Th2 and Th2/1 cells offers a way of interfering with atopic disorders and optimizing immune reactions to helminths/vaccine candidates needs further examination in experimental models.

IMM-O-15

Schistosome egg antigens, including the glycoprotein IPSE/alpha-1, trigger the development of regulatory B cells

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Introduction: Infection with the helminth *Schistosoma mansoni* drives the development of interleukin (IL)-10-producing regulatory B (Breg) cells in mice and man. Breg cells have the capacity to reduce experimental allergic airway inflammation and are thus of high therapeutic interest. However, both the involved antigen and cellular mechanisms that drive Breg cell development remain to be elucidated.
Objectives: To reveal the mechanism of schistosome-induced Breg cell induction, we investigated whether *S. mansoni* soluble egg antigens (SEA) directly interact with B cells to enhance their regulatory potential, or act indirectly on B cells via SEA-modulated macrophage subsets.
Methods and Results: *S. mansoni* eggs or SEA injected intraperitoneally in mice significantly upregulated IL-10 and CD86 expression by marginal zone B cells. SEA was efficiently bound by both B cells and macrophages of the splenic marginal zone *in vivo*, but macrophages were dispensable for Breg cell induction as shown by macrophage depletion with clodronate liposomes. By using pHrodo-labeled SEA, we demonstrated its uptake into acidic compartments of B cells. IL-10 expression was dependent on endosomal acidification and further enhanced by CD40 ligation. Notably, IPSE/alpha-1, one of the major antigens in SEA, was capable of inducing IL-10 in naïve B cells, which was reproduced by tobacco plant-derived recombinant IPSE. Other major schistosomal antigens, omega-1 and kappa-5, had no effect. SEA likely contains more Breg cell-inducing components, because SEA depleted of IPSE/alpha-1 was still able to induce Breg cells. Importantly, SEA- and IPSE-induced Breg cells triggered regulatory T cell development *in vitro*. SEA and recombinant IPSE/alpha-1 also induced IL-10 production in human CD1d+ B cells (Haeberlein et al., PLoS Pathogens 2017).
Conclusion: The mechanism of *S. mansoni*-induced Breg cell development involves a direct targeting of B cells by SEA components such as the secretory glycoprotein IPSE/alpha-1.

IMM-O-16
Host determinants of susceptibility to *Giardia* infection

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Introduction: *Giardia* infection is a common cause of diarrhea in humans with a prevalence of up to 30% in developing regions. Th17 responses and IgA are important for host protection. We have previously identified eosinophils as important supporters of IgA class switching in B cells. Hence, we asked if eosinophil deficiency results in hampered control of *G. muris* infection. Second, we assessed if inbred mouse lines differ in *Giardia* control due to their disparate propensity for the generation of Treg/Th17 responses.
Objectives: The role of eosinophils in supporting IgA responses during *Giardia* infection was monitored in wild type and eosinophil-deficient dblGATA-1 mice. To gain insight into the basis for the varied susceptibility of mouse lines to *Giardia*, the intestinal Th17/Treg balance was surveyed in BALB/c and C57BL/6 mice.
Materials & Methods: Immune responses were surveyed in gut and associated lymphoid tissues. The composition of the gut microbiota was assessed by bacterial 16s rRNA-gene based qPCR.
Results: Compared to BALB/c, naïve dblGATA-1 mice had low intestinal IgA and Th17 levels. Upon infection, IgA+ B cells and Th17 cells increased only transiently, correlating with higher cumulative cyst shedding by dblGATA-1 compared to BALB/c mice. dblGATA-1 mice harboured few segmented filamentous bacteria important for homeostatic intestinal Th17 responses. Co-housing of dblGATA-1 with BALB/c mice partially restored immune responses and cyst shedding in eosinophil deficient mice. Comparing the more resistant C57BL/6 to susceptible BALB/c mice we found that BALB/c mice expand intestinal Treg during acute giardiasis.
Conclusion: Eosinophil-deficient mice mount poor immune responses to *G. muris* infection. If this defect results from the lack of eosinophils or relates to microbiota alterations is addressed in ongoing studies. Furthermore, susceptibility to *Giardia* infection might be determined by the effector/regulatory T cell ratio during infection.

Molecular genetics

MOL-O-01
Analysis of individual histone modifications using CRISPR-Cas9

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Histones can be post-translationally modified by combinations of methylations, acetylations or phosphorylations at different amino acids, thereby serving as recruiting platforms for a multitude of proteins that can influence many biological processes. The general distribution of these histone marks is conserved throughout the eukaryotic kingdom but the *in vivo* role of individual modifications is still poorly understood in any organism. This is due to the fact that histones are encoded by multigene families and that tools to precisely and simultaneously edit multiple genomic loci only recently became available.
The efficiency and precision of CRISPR-Cas9 make this technology ideally suited to edit multicopy

genes, such as histones. Thus, we established this technique in *Trypanosoma brucei* and exploited the fact that DNA breaks are mostly repaired via homologous recombination. To achieve this, we chose an episome-based version in order to edit wild type cells without the insertion of resistance marker and without changing other regions of the genome including the genetic environment of the target locus. Using the episome-based system we were able to (1) insert a gene coding for a fluorescent tag between the target ORF and its 3´UTR, (2) delete both alleles of one gene in only one transfection and (3) precisely edit an individual codon in a multicopy gene array. Most importantly, all of these genome editing processes were achieved without the generation of any detectable off-target effects.
As a proof of principle, we successfully exchanged the lysine residue at position 4 in histone H4 (H4K4) by an arginine (H4R4), thereby mimicking the non-acetylated state. At the end of the selection period more than 90% of the estimated 42 H4 genes were successfully edited.
-The approach opens up the possibility to scrutinize the importance of individual histone modifications for eukaryotic epigenetics.

MOL-O-02
Comparative analysis of different gene editing techniques in *Leishmania donovani*

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Introduction: For the past 20 years researchers relied on homologous recombination (HR) to study reversible genetics in *Leishmania* spp.. Recent developments i.e. CRISPR/Cas9- and DiCre-mediated gene editing allow rapid creation and immediate induction of null mutants, respectively. These new techniques have to be evaluated in comparison to HR. Therefore, I performed a comparable study using HR, CRISPR-Cas9 and DiCre to analyse the four different 60kD chaperonins (CPN60) of *Leishmania donovani*.
Previous studies on CPN60 showed that CPN60.1 expression was below detection, while CPN60.2 (Schlüter et. al., 2000) and CPN60.3 (Silverman et. al., 2010b) were detected on the protein level. The latter two were also found to be part of the immune-modulatory exosomes, but little more is known about the role of CPN60 proteins in *Leishmania*.
Methods: The genes of interest were replaced using either homologous recombination or CRISPR-Cas9. Resulting null mutants were phenotypically analysed for specific changes, such as growth under different conditions, morphology and infectivity. For essential genes, inducible DiCre recombination was used.
Results: HR showed that CPN60.4 and CPN60.2 are non-essential genes in promastigotes. While CPN60.2 and CPN60.4 null mutants did not show any specific phenotype under normal growth conditions, the adjacent gene for CPN60.3 (83% identity) was found to be essential in promastigotes, as was CPN60.1. Results for CPN60.3 and CPN60.4 were confirmed using CRISPR-Cas9. In contrast, generation of viable CPN60.2 null mutants was not possible, while CPN60.1 null mutants were obtained. Currently DiCre-mediated deletion of CPN60.3 is being tested.
Conclusion: The comparative analysis of different gene editing techniques in Leishmania donovani revealed off target effects for homologous recombination and its limitation in creating null mutants for proteins below the detection level. On the other hand DiCre provides a new tool to analyse essential genes.

MOL-O-03

Histone acetylation and histone variants during transcription initiation in *Trypanosoma brucei*

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Question: Histone acetylation is a key regulator in inducing chromatin rearrangements. It mediates nucleosome destabilization by firstly decreasing the DNA-histone interaction within a nucleosome and secondly by inducing histone variant incorporation. Therefore, it is often found in regions of active transcription in many eukaryotes.
In *Trypanosoma brucei*, histone lysine acetylation and the incorporation of the histone variant H2A.Z are major features of transcription initiation sites. Since the parasite's genome lacks canonical promoter motifs, it has been suggested that differences in chromatin structure regulate DNA accessibility and control transcription. However, whether these chromatin marks are involved in transcription initiation has not been proven.
Methods: Using quantitative mass spectrometry and different next generation sequencing approaches we have found that the two essential histone acetyltransferases (HATs) HAT1 and HAT2 act specifically on transcription start site (TSS) histones.
Results: HAT1 changes the acetylation levels of the TSS-specific histone variants H2A.Z and H2B.V. Depletion of HAT2 changes the acetylation pattern of the TSS-nucleosome and is thereby affecting H2A.Z incorporation at these genomic sites. Furthermore, depletion of either HAT1 or HAT2 leads to a strong deregulation of transcription.
Conclusions: Taken together, the accurate quantification of histone acetylation allowed us to determine the function of HAT1 and HAT2 at TSSs and their role in H2A.Z deposition. Additionally, our results indicate that the loss of TSS acetylation affects transcription initiation. Our findings support the hypothesis that histone acetylation and the presence of the histone variant H2A.Z are directly involved in mediating correct RNA polymerase II recruitment to TSSs.

MOL-O-04

Ribosome Profiling Reveals the Role of HSP90 in Stage-specific Protein Synthesis in *Leishmania donovani*

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The 90 kD heat shock protein of *Leishmania donovani* is essential for proliferation and plays a pivotal role in promastigote-to-amastigote stage conversion: inhibition of HSP90 causes growth arrest, a stress response and expression of amastigote-specific marker genes. HSP90 is also subject to amastigote stage-specific phosphorylation, further implicating this molecular chaperone in the control of the parasite's life cycle. Here, we describe a Ribosome Profiling analysis of *L. donovani,* comparing protein synthesis patterns in the presence and absence of active HSP90, covering 98% of the known

protein coding genes. Firstly, we find that ribosome-protected RNA faithfully maps the open reading frames of *L. donovani* genes. Secondly, no correlation was found between RNA steady state levels quantified by RNA-Seq and protein synthesis rates, confirming a regulated translation as a means for gene expression control. Thirdly, inhibition of HSP90 affects the synthesis of metabolic enzymes, effecting a shift from carbohydrate to amino acid and fatty acid metabolism, strongly resembling the metabolic shifts observed by proteome analysis during axenic stage conversion (Rosenzweig et al., 2008). Fourth, inhibition of HSP90 triggers increased synthesis of stress response proteins, matching earlier, protein-specific analyses. Lastly, inhibition of HSP90 causes a significant upshift of protein synthesis, indicating a more general function in the control of translation. Our data therefore demonstrate a role for the major chaperone HSP90 in the regulation of both stage-specific and general protein synthesis and the suitability of Ribosome Profiling to unravel highly complex gene expression pathways.

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MOL-O-05
Six PX domains of *Giardia lamblia* exhibit diverse phosphoinositide-binding profiles

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Introduction: Phosphoinositides (PIPs), the key regulators of membrane trafficking events, are recognized by some lipid-binding domains, such as PX. PX domains bind to different lipid ligands and these proteins have diverse cellular functions.
Objectives: The endomembrane system of *Giardia* is significantly different from that of most model eukaryotes. This system is vital for the trafficking of cyst wall proteins during encystation. As membrane lipids are key regulators of this system, this study aimed to identify and characterize the ligand-binding specificity of the PX domains of *Giardia*.
Materials & methods: BLAST search with yeast PX domains identified six PX domain-encoding genes in *Giardia*. Both *in vitro* biochemical and *in vivo* localization assays were used to determine the ligand specificity of these domains. Real-time PCR was used to monitor changes in the expression patterns of the genes during encystation.
Result: Although all the six PX domains possess almost all the conserved motifs of canonical PX domains, two of six PX domains have non-canonical amino acid residues in their ligand-binding pocket. Sequence analysis shows that the ORFs contain only PX domains and no other associated domains. Based on *in vitro* and *in vivo* approaches it was observed that the PX domains display diverse PIP-binding spectrum. Real-time PCR analyses indicate variation in the expression pattern of these genes in trophozoites, encysting trophozoites and cysts.
Conclusion: The differential expression pattern of the six genes during encystation indicates that the proteins may be involved in diverse cellular pathways. The diversity of the ligand-binding spectrum of

the six PX domains implies that although the endomembrane system of *Giardia* appears to be simple in morphological terms, there is considerable complexity in terms of the lipid distribution. This is the first study to document the presence of sulphur-containing amino acid residues in the ligand-binding pocket of PX domains.

MOL-O-06
Minimal ESCRT machinery of *Giardia lamblia*

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Introduction: At different subcellular locations, the ESCRT pathway induces negative curvature in membranes. This pathway has been studied largely in model eukaryotes belonging to Opisthokonta. However, many nonopisthokont genomes may encode fewer ESCRT components. One of the most minimal set of ESCRT components was identified in the excavate *G. lamblia*.
Objective: This study investigated the functional divergence between the yeast and giardial ESCRT components.
Materials & methods: BLAST search of the *Giardia* genome identified its ESCRT orthologues. Reverse transcriptase PCR was done to check the expression of the identified genes in both trophozoites and cysts. Immunofluorescence was used to localize two of the ESCRT proteins in trophozoites. Functional complementation was carried out in yeast. Interactions within various ESCRT complexes were analysed with either two-hybrid assay or by monitoring the distribution of GFP-tagged segments of the two paralogues of GlVps46, a ESCRT-III component.
Results: The ESCRT pathway of *Giardia* most likely operates at the peripheral vesicles, its endolysosomal compartments, as multiple components localize there. While *Giardia* encodes multiple orthologues of some ESCRT components, searches failed to identify those for many others. Orthologues of Vps25, Vps2, Vps46 and Vps4 can substitute for the corresponding yeast proteins, but those for Vps22, Vps20 and Vps24 cannot. In comparison to that of yeast, while some of the binary interactions have been preserved within the ESCRT-II complex, others have not. The two Vps46 paralogues appear to have diverged in terms of their interaction with Vps24 and Vps2, with one being more similar to that of yeast.
Conclusion: While certain interactions within the ESCRT–II and –III complexes have been preserved in yeast and *Giardia*, others have been altered considerably during the course of evolution. Results of this study show that the mechanism of ESCRT complex assembly may not be universal.

Parasitic helminths

PAH-O-01

Genome wide expression profiling of the *Echinococcus multilocularis* stem cell system

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It has been shown that the only proliferating cells in *Echinococcus multilocularis* are undifferentiated stem cells (so called "germinative cells"). However, little is known about the overall transcriptome of these germinative cells. In this work, we studied the germinative cells of the fox tapeworm *Echinococcus multilocularis* through genome wide expression profiling. We used three approaches to identify genes that are specifically expressed in germinative cells. Metacestode larvae were depleted of germinative cells by treatment with either hydroxyurea or the polo-like kinase inhibitor Bi2536. With differential expression analysis of the transcriptome we identified genes with significantly lower expression in samples depleted of germinative cells compared to control samples. In a third approach, we compared the transcriptome of early primary cell cultures, which are enriched in germinative cells, to later stages of primary cell culture, which contain less germinative cells. Genes with higher expression in the early primary cell culture are likely expressed in germinative cells. Genes that could be identified by all three approaches were considered to be specifically expressed in germinative cells. To validate the results, we performed quantitative RT-PCR and whole mount in situ hybridizations for selected genes. More than 600 genes are specifically expressed in germinative cells. These genes are coding for transcription factors, proteins for DNA repair and DNA replication, as well as known proliferation markers and known markers of Echinococcus multilocularis germinative cells. Quantitative RT-PCR for selected genes showed lower expression in samples depleted of germinative cells than in control samples. Whole mount in situ hybridization e.g. for the telomerase gene showed expression in germinative cells. The knowledge of genes that are specifically expressed in germinative cells will facilitate future research on the *Echinococcus multilocularis* germinative cells.

PAH-O-02

Exploratory metabolomics study of the experimental opisthorchiasis in a laboratory animal model (golden hamster, Mesocricetus auratus)

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Background: Opisthorchiasis is a parasitic infection caused by the liver flukes of the Opisthorchiidae family. Both experimental and epidemiological data strongly support a role of these parasites in the etiology of the hepatobiliary pathologies and an increased risk of intrahepatic cholangiocarcinoma. Understanding a functional link between the infection and hepatobiliary pathologies requires a

detailed description a host-parasite interaction on different levels of biological regulation including the metabolic response on the infection. The last one, however, remains practically undocumented. Here we are describing a host response on Opisthorchiidae infection using a metabolomics approach and present the first exploratory metabolomics study of an experimental model of O. felineus infection.
Methods and Findings: We conducted a Nuclear Magnetic Resonance (NMR) based longitudinal metabolomics study involving a cohort of 30 animals with two degrees of infection and a control group. An exploratory analysis shows that the most noticeable trend (30% of total variance) in the data was related to the gender differences. Therefore further analysis was done of each gender group separately applying a multivariate extension of the ANOVA-ASCA (ANOVA simultaneous component analysis). We show that in the males the infection specific time trends are present in the main component (43.5% variance), while in the females it is presented only in the second component and covers 24% of the variance. We have selected and annotated 24 metabolites associated with the observed effects and provided a physiological interpretation of the findings.
Conclusions: Our data show that at early stage of infection a response of an organism unfolds in a gender specific manner. The physiological mechanisms of a response are best described as a state of metabolic stress.

PAH-O-03

Forecasts of attaining onchocerciasis elimination in Ogun State, Nigeria; a cross-sectional report of the Ov-16 serology (Rapid Diagnostic Test and ELISA) among children born after 10 years of treatment with ivermectin

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Background: Evidence-based studies using the Ov-16 serology among children below 10 years of age is one of the key components of the revised WHO course of action for indicating interruption of transmission of *O. volvulus* among human population receiving treatment with ivermectin.
Methods: In view of this background, this study conducted between March and July 2015 investigated the sero-prevalence of onchocerciasis in endemic communities of Ogun State, Nigeria after 10 years of treatment with ivermectin. Using the Ov16 Rapid Diagnostic Test (RDT), 719 children between the age 5-9 years residing in 32 firstline communities in 8 endemic Local Government Areas (LGA"s) provided whole blood specimen which was tested for IgG4 antibodies against the *O. volvulus* antigen Ov-16. Data were analyzed using Pearson"s Chi-square in SPSS 20.
Results: Results showed a cumulative sero-prevalence of 21(2.9%), Relationship between age and prevalence was statistically insignificant ($p > 0.05$). Thirteen females and eight males were exposed to *O. volvulus* respectively. The low sero-prevalence recorded among children within the age range (5-9 years) born after the inception of ivermectin implementation implies that they may have had diminutive historic exposure. Although this finding is somewhat greater than the 0.1% threshold set by the guideline for this study population.

Conclusions: The Information obtained will serve as a baseline serological information and a guide to prepare Ogun State for Post-Treatment Surveillance (PTS) AND Transmission Assessment Survey (TAS) in the nearest future.

PAH-O-04

Histopathological changes during the course of *Toxocara canis*- and *T. cati*-induced neurotoxocarosis

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Zoonotic roundworms of the genus *Toxocara* may lead to several forms of disease in humans, including neurotoxocarosis (NT). Previous studies on *T. canis*- and *T. cati*-induced NT in the model organism "mouse" have indicated pathological changes and clinical symptoms comparable to human NT. The objective of this study was to provide an extensive histopathological characterization, comparing *T. canis*- and *T. cati*-induced NT in mice over the course of infection. Five brains each of *T. canis*- and *T. cati*-infected as well as uninfected mice were investigated 7, 14, 28, 42, 70 and 98 days post infectionem (dpi), while brains of *T. cati*-infected and control mice were also available from 120 and 150 dpi. Two histological sections of cerebra and cerebella, respectively, were stained with haematoxylin-eosin. Demyelination was visualized by Luxol fast blue staining. Immunohistochemistry was carried out to study microglia cell morphology and to detect accumulation of amyloid precursor protein, an indicator of axonal damage. Haemorrhages, haemosiderophages, eosinophilic vasculitis and activated microglia were detected in both infection groups starting 7 dpi, followed by eosinophilic meningitis in cerebra 14 dpi. Degenerative processes (demyelination, vacuolization, gitter cells) were detected earlier (14 vs. 70 dpi) and in a larger proportion for *T. canis*- than *T. cati*-infected mice. Those degenerative processes intensified over time. During *T. canis*-infection, larvae were observed in cerebella, corpus callosum and cerebral cortex, whereas during *T. cati*-infection, larvae were found mainly in cerebella. Nevertheless, changes were more severe in cerebella than in cerebra in both infection groups. The more pronounced pathology during *T. canis*- than *T. cati*-NT in mice is in accordance with more severe behavioral changes previously observed in this infection group. Stronger affinity of *T. canis* larvae to the CNS, beside diverse regulatory mecahnisms, may contribute to this phenomenon.

PAH-O-05
Concurrent helminth infection interferes with vaccine-induced protection against influenza virus infection

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Introduction: Parasitic helminths infect 2 billion people worldwide thereby dampening the immune system of their hosts. Accordingly, several studies report a negative correlation between pre-existing helminth infection and response to vaccination in the human population. We have reiterated these findings in mice by showing that concurrent infection with the parasitic nematode *Litomosoides sigmodontis* suppressed the antibody (Ab) response to model antigen vaccination.
Objectives: We aim to analyse the clinical relevance of helminth-induced interference with Ab response to vaccination.
Material and Methods: We vaccinated non-infected and *L. sigmodontis*-infected mice with the commercially available seasonal (2015/16) trivalent subunit anti-influenza vaccine Begripal (Seqirus), that is licensed for humans. We quantified HA-specific Ab responses and recorded weight loss and virus burden upon challenge infection with the human pathogenic influenza strain H1N1 A/Hamburg /NY1580/09.
Results: Mice that were infected with *L. sigmodontis* for 4 weeks at the moment of vaccination displayed reduced titres of HA-specific IgG as well as a reduced neutralizing capacity compared to non-infected mice. Reduced Ab response in helminth-infected mice was reflected by increased weight loss and increased influenza virus burden during challenge infection compared to vaccinated, non helminth-infected mice. Of note, also mice that were vaccinated several months after immune-driven termination of *L. sigmodontis* infection displayed reduced Ab responses compared to the non-infected age matched control group. Repeated vaccination or vaccination with an adjuvanted anti-influenza vaccine elevated the Ab response in helminth-infected and non-infected mice in general, but did not abrogate the helminth-induced suppression.
Conclusion: Our results suggest that anti-influenza vaccination may be less protective in individuals with either acute or a history of previous helminth infection.

PAH-O-06
Host-parasite-microbiota interactions in thoroughbred horses

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A growing body of evidence supports the existence of a complex network of interactions occurring between gastrointestinal (GI) helminth parasites and the gut commensal bacteria, with substantial effects on both host immunity and metabolic potential. However, little is known of the fundamental biology of such interactions in veterinary species. Given the considerable economic losses associated with GI helminths, particularly in livestock and equines, as well as the global threat of emerging anthelmintic resistance, further explorations of the complexities of host-helminth-microbiota

interactions in these species are needed. This study characterises the composition of the equine bacterial commensal flora associated with the presence of low (C*low*) - and high (C*high*) numbers of eggs of an important group of equine GI parasites (i.e. the cyathostomins), prior to and following anthelmintic treatment. High-throughput sequencing of microbial 16S rRNA amplicons and associated bioinformatics and statistical analyses of sequence data revealed strong clustering according to faecal egg counts (P = 0.003). A trend towards increased populations of Methanomicrobia (class) and *Dehalobacterium* (genus) was observed in C*low* in comparison to C*high*. Anthelmintic treatment in C*high* was associated with a significant reduction of the bacterial phylum TM7 14 days post-ivermectin administration, as well as a transient expansion of *Adlercreuzia* spp. at 2 days post-treatment. This study provides a first insight into the intimate mechanisms governing host-parasite-microbiota interactions in equids, and sets a basis for the development of novel, biology-based intervention strategies against equine GI helminths based on the manipulation of the commensal gut flora.

Ethical Declarations:
Ethical Animal Research: The authors declare that ethical approval for this project has been granted by the University of Cambridge, Research Ethics Committee.
Competing Interest: The authors declare that they have no conflict of interest in relation to this work.
Funding Source: This work was funded by the Horserace Betting Levy Board, The Thoroughbred Breeder"s Association and the British European Breeders Fund.

Parasite-host interactions I

PHI-O-01

Giardia duodenalis infection of human intestinal cells: Modelling asymptomatic colonization?

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Giardia duodenalis is 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, enigmatic to giardiasis is the range of medical conditions from severe chronic enteritis to complete asymptomatic courses. We investigated whether *G. duodenalis* trophozoites can compromise epithelial barrier function by using an *in vitro* co-culture system with Caco-2 monolayer. Complementary experiments using human small intestinal cells derived from organoids are currently being carried out for comparison.
Measurements of trans-epithelial electric resistances (TEER) on *Giardia*-infected Caco-2 monolayers in transwell culture assays were used to indicate epithelial permeability and alterations of tight junctions were analyzed via immunofluorescence assays (IFAs). Additionally, the release of several cytokines was investigated via Luminex® assay. *Giardia* trophozoites were isolates from symptomatic patients and grown axenically until testing. Testing 11 *G. duodenalis* isolates (A1, A2, B, E assemblages) revealed a reproducible dose-dependent and attachment-independent TEER-increase in parasite-Caco-2 co-cultures. This effect was uniform irrespective of culture conditions (aerobic/anaerobic, various incubation time, virus-infected isolates) or applied infection doses. Furthermore, no degradation or

delocalization of several tight junction proteins was observed. Also, cytokine levels were unchanged upon *G. duodenalis* infection. Primary human intestinal epithelial cell culture monolayers derived from intestinal organoids have been set up for comparative experiments. Our data suggests that the widely used *G. duodenalis/*Caco-2 co-culture model recapitulates the course of asymptomatic infections and additional, yet unknown factors may be required to induce barrier dysfunction. We currently test the hypothesis that organoid-derived primary cell culture monolayers will represent a similarly or better suitable *in vitro* model to elucidate the mechanisms of *G. duodenalis* pathogenesis.

PHI-O-02

Trypanosoma cruzi infection mediates phosphoproteomic networks associated with pathology in colonic epithelial cells

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Introduction: The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease, a neglected tropical disease which causes severe morbidity and mortality in afflicted individuals. The disease which was originally endemic in Latin American countries has now become a new global health problem. Currently, the disease is present in all major economically advanced countries worldwide due to inevitable modern globalization. About 30% of *T. cruzi* infected individuals eventually present with cardiac, gastrointestinal tract and/or neurological disorders. Megacolon, one of Chagas disease major pathologies, is accompanied by gastrointestinal motility disorders that have been attributed to alterations in the number of interstitial cells of Cajal and enteric nervous system defects.
The objective of our study is to delineate the molecular mechanisms of *T. cruzi* induced megacolon which will pave the way for the development of modern intervention strategies.
Materials and methods: We challenged primary human colonic epithelial cells with invasive *T. cruzi* trypomastigotes and evaluated the modulation of intracellular phosphoproteins using the Proteome Profiler Human Phospho-Kinase Array Kit (R&D). Data obtained was used to map the associated networks that could be responsible for the observed pathology.
Results: We observed that early during the process of *T. cruzi* infection, the parasite induces modulations in the phosphoprotein levels in host cells that lead to changes in the phosphoproteomic pathways. These changes result to an increase in the activation of several transcription factors including c Jun and CREB validated by immunoblotting and immunocytochemistry.
Conclusion: *T. cruzi* dysregulates colonic epithelial cell phosphoproteomic patterns early during infection. The pathogen induced phosphorylation profile is tilted towards the induction of inflammatory and fibrogenic responses which are potential hallmarks of megacolon pathology.
Supported by NIH grants: 1SC1AI127352-01A1, G12MD007586 and Meharry Translational Research Center (MeTRC) (5U54MD007593-09)

PHI-O-03
African Trypanosomes Can Evade Immune Clearance by Sugar-Coating Antigenic Surfaces

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Trypanosoma brucei subspecies cause sleeping sickness in humans and nagana in livestock. The parasites elicit a robust antibody-mediated immune response to their exposed Variant Surface Glycoprotein (VSG) coat but evade host immunity by repeatedly accessing a large genetic VSG repertoire and "switching" to antigenically distinct coats. The 1.4Å resolution crystal structure of variant VSG3 manifests heretofore unappreciated divergence in the tertiary fold and oligomeric states of these surface molecules. The structure also reveals an *O*-linked carbohydrate on the uppermost surface of the VSG, a modification previously unknown in African trypanosomes. Mass spectrometric analysis indicates that this *O*-glycosylation site is heterogeneously occupied by 0 to 3 hexose residues in VSG3 and is also present in other VSGs. Antibody binding and infection assays demonstrate the functional relevance of this modification, which potently impairs immune recognition and pathogen clearance. These data suggest unanticipated VSG variability at the post-translational level with consequences for immune evasion.

PHI-O-04
Impact of naturally acquired immunity to *Plasmodium falciparum* on *var* gene expression in controlled human malaria infections

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Question: The virulence of *P. falciparum* has been linked to the variant surface antigen *Pf*EMP1 (erythrocyte membrane protein 1), which is encoded by 60 *var* genes per parasite genome. The rate and mechanism at which parasites switch *Pf*EMP1 expression to undergo antigenic variation is of tremendous interest and controlled human malaria infections provide a unique opportunity to further determine *var* gene expression patterns *in vivo*.

Methods: We analyzed expression of the entire *var* gene repertoire in *ex vivo* parasites sampled either from malaria-naïve or from infected volunteers with lifelong malaria exposure. Furthermore, the antibody recognition of *Pf*EMP1 domains responsible for particular binding phenotypes of infected erythrocytes was assessed.
Results: The parasite population in each malaria-naïve volunteer expresses all or most *var* gene variants, but transcription was predominantly from the subtelomeric located *var* gene groups A and, particularly, B. In contrast, lifelong malaria-exposed volunteers show reduced parasite multiplication, hardly detectable expression of A-type *var* genes and can be categorized into "non-controllers" and "controllers". Parasites from "non-controllers" with low pre-existing *Pf*EMP1 antibody levels show an exponential parasite growth and a broad *var* gene expression pattern of mostly B-type *var* genes. In "controllers" higher *Pf*EMP1 antibody levels are associated with a suppressed parasite replication rate and their parasites express only one or very few B or B/C-type *var* gene variants on a population level.
Conclusion: These data indicate that *P. falciparum* parasites express a broad repertoire of subtelomeric *var* genes at the onset of blood stage infection, which in naïve hosts enable it to efficiently explore the available host cell receptor landscape for sequestration. However, in semi-immune individuals the pre-existing immunity limits the survival of parasites to the subpopulations, which express non-recognized *Pf*EMP1 variants.

PHI-O-05
Changes in the transcriptome of human brain endothelial cells (HBECs) in response to *Plasmodium falciparum* malaria infection

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Introduction: In its life cycle *P. falciparum* invades erythrocytes. To avoid the clearance in the spleen, the parasite developed a unique mechanism, called cytoadherence, in which infected erythrocytes (IEs) adhere to endothelial cells (ECs). In microvessels IEs can block the bloodstream and lead to hypoxia, tissue damage and organ failure. If IEs bind to ECs in the brain, cerebral malaria (CM) occurs. Cytoadhesion also induced inflammatory response and endothelial dysfunction, which contributes the severity of a CM.
Objectives: In this study, we are investigating the transcriptional changes of HBECs and parasites in response to their binding. In this context, it seems highly probable that HBECs can respond to IE adhesion with secretion of different chemokine and cytokines. Thus, we also want to search for transcriptional changes as well as changes in the cytokine/chemokine profile of the HBEC after exposure to blood plasma of *P. falciparum*-infected patients and IEs.
Material and Methods: The transcriptomes will be generated by using the Illumina-HiSeq 2500/4000 platform. The determination of the soluble cytokine/chemokine profile is performed by using BioLegend LEGENDplex bead-based immunoassay.
Results: First transcriptional analysis shows that cytoadhesion and the exposure to patient"s plasma triggers approximately 1000 genes in HBEC. Large quantities of these genes are associated with inflammatory response. These transcriptional changes are mainly mediated via Tumor necrosis factor-signaling and NF-kappa B signaling pathway.
Conclusion: The generated data of the whole transcriptome of HBECs in response to IEs cytoadhesion will give us new insights of pathogenic factors of *P. falciparum*. By including soluble cytokines and

chemokines as pathogenicity factor of a CM, we are getting closer to the pathogenic conditions within an infected human body.

PHI-O-06

Association of TNF-α -308G/A polymorphism with *Plasmodium falciparum* and *Schistosoma haematobium* infections in Nigerian children

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This study investigated the association between Tumor Necrosis Factor-alpha (TNF-α)-308G/A gene polymorphism, an important human cytokine and susceptibility to *P. falciparum* and *S. haematobium* infection among school children in Nigeria. Blood samples collected from 394 school age children, comprising of 105 single asymptomatic *P. falciparum*, 104 single *S. haematobium* and 68 co-infected with *P. falciparum* and *S. haematobium* and 117 who were uninfected with either pathogen were analyzed. Polymorphism of TNF-α-308G/A was genotyped using Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR). The frequency of the genotype TNF-α -308GG was significantly higher in *S. haematobium* infected children when compared to uninfected children (HC vs SH; p=0.006; OR=3). In addition, the frequency of the A allele was significantly higher in *S. haematobium* infected children compared to the healthy control (HC vs SH; p=0.007; OR = 1.7). For the comparison of co-infection of *P. falciparum* and *S. haematobium* versus healthy control, no significant difference was observed. We observed a significant difference between *P. falciparum* parasitemia and TNFα-308 gene with GA genotype having higher a frequency (p=0.002). For *S. haematobium*, the comparison of genotypes showed heterozygous GA to be significantly more associated with egg count when compared to GG and AA (p=0.0011) and to AA alone (p=0.033). Our data showed that TNF-α -308AA genotype and A alleles could be a susceptible factor in acquisition of *S. haematobium* infection. Additionally, the TNF-α -308G/A polymorphism appear to contribute to increase *P. falciparum* parasitemia and *S. haematobium* egg in Nigerian children

PHI-O-07

A cell culture-based system to assess the role of Immunity-Related GTPases in the maintenance of virulent *Toxoplasma gondii* strains in wild rodents

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Introduction: Resistance to infection with virulent *T. gondii* strains relies on the IFN-γ-induced polymorphic Immunity-Related GTPase *Irgb2-b1* in *Mus musculus*, providing an explanation for the maintenance mechanism of virulent strains in nature. However, in Europe cats – the definitive host for *T. gondii* – prey more on other rodent species, e.g. *Myodes glareolus*, *Microtus* spp. and *Apodemus*

spp. They also show higher *T. gondii* seroprevalences compared to *Mus* spp., implying they could be more relevant intermediate hosts.
Objectives: We aim at assessing whether specific IRGb2-b1-like proteins confer resistance to virulent *T. gondii* infection in these species, similar to *Mus* spp.
Material & Methods: We devised a strategy that allows us to efficiently PCR amplify, clone, sequence and then express wild rodents" *Irgb2-b1*-like genes in lab mice-derived fibroblasts. The latter allow growth of avirulent but not of virulent *T. gondii* strains upon IFN-γ treatment. Following infection with virulent *T. gondii* we can then observe whether the introduced sequences lead to parasite death.
Results: As reference we cloned *Irgb2-b1*-like cDNAs of cell lines from *M. glareolus*, *Microtus arvalis* and *Apodemus agrarius,* after induction with either our custom-produced recombinant vole IFN-γ or commercial mouse IFN-γ, respectively. In addition, using genomic sequences from samples of a large collection across Germany of *M. glareolus*, *Microtus* spp. and *Apodemus* spp. tissue samples our first results indicate substantial amino acid diversity between and also within these species. Based on the cDNA sequences from the rodent cell lines we created stable Flp-InTM-3T3 cell clones expressing IRGb2-b1-like proteins that we showed localized to the PVM of virulent parasites. We are currently analyzing the resulting phenotypes upon infection with virulent and avirulent *T. gondii*.
Conclusion: 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*. They will help to assess the ecological importance of wild rodents as intermediate hosts for virulent parasite transmission to cats.

PHI-O-08
House mouse hybrids show higher vigour in response to *Eimeria* infection compared to pure (sub-)species

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Parasites have been suggested to play a role in hybrid zones. Health differences when confronted with infections (immunological vigour) could offset reductions in fertility, that is: increased health could counterbalance other fitness components and thereby reduce the "net strength" of isolating barriers. Further, hosts can resist or tolerate parasite load (parasitemia). Thus disentangling host fitness, health and parasitemia, all in the context of parasites optimising their own fitness, requires care. We focus on host health and parasite fitness (HH, PF) proxies in a complementary approach: large scale field sampling in the European House Mouse Hybrid Zone between *Mus musculus domesticus*, *Mus musculus musculus* and laboratory infection experiments with *Eimeria* spp., a host-specific intracellular apicomplexan.
In the field, parasitemia (PF) is higher in "pure" hosts than hybrids. Distinguishing between three species of *Eimeria*, we find evidence for parasite-"pure"-host adaptation contributing to this pattern. In a laboratory experiment, we find parasite reproductive output (PF) to be higher in *M. m. musculus*

compared to hybrids and *M. m. domesticus*. Weight retained (HH), is however higher in hybrids and in *M. m. musculus* than in *M. m. domesticus*.
We argue that parasitemia and host health should be studied together, each with its appropriate measures, and that host health is likely a better proxy for the immunological component of host fitness than parasitemia, which, like parasite reproductive output, is more suited as a (PF) proxy for parasite fitness. A system allowing these joint observations in the laboratory and in the field is optimal. Discrepancies between previous studies can be reconciled with this approach, which allows us to conclude that when confronted with parasite infections, hybrids show increased vigour compared to "pure" mice, while simultaneously parasite reproduction is more effective in a parasite-host adapted combination involving one of the "pure" host taxa.

PHI-O-09
What can we learn about the function of the key *Toxoplasma gondii* virulence effector protein IST in closely related *Hammondia hammondi* and *Neospora caninum*?

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Introduction: *Toxoplasma gondii* is one of the most successful parasites in the world, with a broad host range that includes virtually all warm-blooded animals. To establish a suitable niche for survival and localization signal (NLS). The functionality of *Hammondia* IST was, however, gained when we added an artificial NLS to the protein sequence. Using truncated protein versions, we were able to identify a small homologous helical domain of < 40 amino acids of *Toxoplasma* IST and surprisingly also *Hammondia* IST sufficient for the IFN pathway blockage, whereas the domain is lacking in *N. caninum*. We further investigated other potential roles of IST and host protein binding partners of the IST protein by a targeted CRISPR/Cas9 approach.
Conclusion: Our findings reveal why *H. hammondi* and *N. caninum* are unable to block the IFN response and might suggest a more diverse role of IST in host cell manipulation, beyond what is currently appreciated.

PHI-O-10
Chronic *Toxoplasma gondii* infection leads to phenotypic changes in human monocytes *in vivo* and *in vitro*

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Toxoplasma gondii is an obligate intracellular parasite that establishes chronic infections in immunocompetent mammals including up to 30% of humans worldwide. Monocytes are importan host cells for *Toxoplasma* during acute infection and are critical regulators and effectors of anti-parasitic immunity during infection. Here, we unravelled phenotypic differences of monocytes from healthy blood donors either chronically infected with *T. gondii* or *T. gondii*-negative, and we determined monocyte responses after parasite infection *in vitro*.

Peripheral blood mononuclear cells from blood donors with chronic toxoplasmosis or from sero-negative controls were enriched for monocytes which were then directly analysed for expression of various cell surface markers or for cytokine mRNAs. Cells were also infected with *T. gondii in vitro* or were left non-infected and were analysed at 24 hours p.i. *Ex vivo* analyses of monocytes revealed a reduced expression of CD16 on monocytes from chronically infected individuals as compared to those from sero-negative controls. Furthermore, the percentages of CD62L+ and CD64+ monocytes were decreased or increased, respectively, in individuals with chronic toxoplasmosis as compared to controls. *In vitro* infection of monocytes from both sero-positive and sero-negative blood donors with *T. gondii* led to an expansion of CD14 single positive classical monocytes and a decrease of CD14/CD16 double positive monocytes. Furthermore, the percentages of CCR2+ monocytes strongly decreased after infection. Finally, expression of IL-12 mRNA increased after infection with *T. gondii* particularly in cells from chronically-infected individuals, but to a lesser extent also in those from sero-negative controls.
Together, these results reveal that chronic toxoplasmosis in humans may exert long-term effects on the phenotype of monocytes, i.e. cells of the innate immune system. These alterations may have important implications for the function of these cells.

PHI-O-11
Inhibition of inflammasome activation in human cells upon *Toxoplasma gondii* infection

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Resistance against infection by *T. gondii* in mice is largely dependent on two families of IFNg-inducible proteins, the Immunity-Related GTPases (IRG proteins) and Guanylate-Binding Proteins (GBP proteins). Accumulation at the parasitophorous vacuole membrane is a prerequisite for parasite and subsequent cell death. *T. gondii* has evolved distinct mechanisms to overcome this cell-autonomous immune mechanism. Two effectors secreted from secretory organelles of the parasite during host cell invasion, ROP18 and ROP5, have been shown to specifically inactivate certain IRG proteins. In human cells, resistance against *T. gondii* is largely mediated by depletion of tryptophan, dependent on IFNg-induced expression of indoleamine 2,3-dioxygenase (IDO). It appears now as if IDO-independent control is mediated by expression of certain hGBP proteins but the mechanism of GBP proteins against *T. gondii* infections in human cells has not been revealed yet. However, in recent studies it was shown that hGBP5 promotes selective NLRP3 inflammasome responses to pathogenic bacteria whereas *T. gondii* is an activator of the NLRP1 and NLRP3 inflammasomes *in vivo*. We aim to decipher the molecular basis of inflammasome activation upon *T. gondii* infection in human cells.
We determined the pro-IL-1β and IL-1β levels in human PBMCs isolated from Colombian patients with ocular, chronic asymptomatic or no signs of toxoplasmosis after infection with virulent *T. gondii* strain RH or RHΔ*rop18*. In the cells of all clinical groups infected with RHΔ*rop18*, higher levels of pro-IL-1β have been detected compared to RH infections. Moreover, an impact of *T. gondii* ROP5 and ROP18 on inflammasome activation could be demonstrated after infection of THP-1 cells. Subsequent biochemical analyses revealed the interaction of certain human GBP proteins with ROP18 and ROP5.
Our data contribute to understand the role of GBP and ROP proteins in inflammasome activation in human *T. gondii* infections.

PHI-O-12
Establishment of *Babesia microti* developmental cycle and its experimental application

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Growing incidence of human infections by protozoan parasite *Babesia microti* defines babesiosis as an emerging disease from the aspect of human medicine and currently the great attention is paid to research of *Babesia*, the tick-transmitted malaria relative. However, the knowledge about interactions between the *Babesia* parasite and its tick vector is still insufficient, particularly due to the absence of quantitative laboratory babesiosis model. We introduce here the fully optimized model of *B. microti* employing infected laboratory mice and immature tick stages of *Ixodes ricinus*. Detailed information about parasite dissemination inside the tick tissues are given by newly implemented visualization and quantification techniques. Special emphasis is paid to parasite stages inside the salivary glands, the primary site responsible for *Babesia* transmission from the vector into the host. Using specific gene silencing we screened the tick immune pathways and evaluated their role in *Babesia microti* acquisition. Overall, our results provide detailed view into mutual molecular and immune interactions between the most common agent of human babesiosis and its tick vector.

Acknowledgement: Supported by GACR grant No. 15-12006Y (VU), *17-27393S (RS), 17-27386S (OH),* GACR 17-14631S (DS)*, and INRA, France.*

PHI-O-13
Murine eosinophils trap microfilariae of the rodent filarial nematode *Litomosoides sigmodontis* in an ETosis-dependent mechanism

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During filarial infection eosinophils mediate protection against adult worms and microfilariae (MF), whereas they are only essential against infective L3 larvae following re-infection or vaccination. Similar to neutrophils, eosinophils are able to produce extracellular DNA traps (ETosis), a form of cell death where intracellular DNA is explosively released. The aim of this study was to analyze the impact of eosinophil ETosis in response to different filarial life cycle stages.
Bone-marrow-derived eosinophils release DNA in response to MF of the rodent filarial nematode *Litomosoides sigmodontis* as was shown by scanning electron and confocal microscopy and reduced MF motility *in vitro* in a DNA- and contact-dependent manner. Subsequent PCR analysis revealed that the released DNA was of nuclear content. In contrast, L3 larval motility was only impaired by eosinophils in the presence of serum from infected wild type animals, but not serum of naive wild type

or antibody-deficient µMT mice, in a partially DNA-dependent and complement independent manner. Trapping of adult worms was only accomplished by eosinophils in the presence of naive serum, but not serum of infected animals. Comparison of the efficacy of bone-marrow-derived eosinophils and eosinophils from the thoracic cavity and gut of *L. sigmodontis*-infected animals further revealed that eosinophils from infected animals are more potent in inhibiting MF motility independent on local immunomodulation by adult worms within the thoracic cavity.
These results demonstrate that eosinophils impair MF motility in an ETosis-dependent mechanism, while L3 larvae are only trapped when specific antibodies are present, as it occurs during filarial re-infection or vaccination. Furthermore, our results suggest that immunomodulatory mediators are present in serum of filarial-infected animals that prevent eosinophil-mediated effects against adult worms.

PHI-O-14
Multiplex profiling of inflammation-related mediators in *Toxocara canis*- and *T. cati*-infected brains

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Toxocarosis, one of the most frequent zoonotic infections worldwide, is caused by somatic migration of *Toxocara canis* and *T. cati* larvae in paratenic hosts. The so-called neurotoxocarosis (NT) is initiated by larvae migrating and persisting in the central nervous system (CNS). Even though modulation of the host"s immune response by *Toxocara* spp. has been demonstrated, detailed data concerning molecular pathogenic mechanisms and involvement of signalling molecules are lacking. Important molecules, involved in the complex molecular signalling network in infection and inflammation, are polyunsaturated fatty acid-derived bioactive regulatory lipids (e.g. oxylipins). To elucidate changes in the oxylipin pattern during the course of *T. canis*- and *T. cati*-induced NT, lipidomic profiling of cerebra and cerebella of experimentally infected C57Bl/6J mice was conducted at six different time points post infection (pi). A total of 74 different eico-/docosanoids were successfully quantified in analysed brains. Only minor changes were detected in the biosynthetic pathway of mostly pro-inflammatory prostaglandins (COX-pathway). In contrast, a significant increase of potentially anti-inflammatory metabolites of the ALOX-pathways was observed for both infection groups and brain regions starting day 14 pi, reaching a peak day 42 pi and declining gradually days 70-98 pi. This may indicate parasite-induced immunomodulatory effects to evade the host"s immune response, facilitating persistence in the brain. To investigate if the brain cytokine milieu is also shifted to a predominantly anti-inflammatory driven immune response during NT, a comprehensive profiling comprising 23 different cyto- and chemokines is currently in progress and results will be presented. The combined data of different inflammation-related signalling molecules during the disease will contribute to the characterization of the still mostly unknown pathogenesis of *T. canis*- and *T. cati*-induced NT.

Focus Session • Physics of parasitism

POP-O-01

The Physics of Parasitism

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Progress in molecular and cellular parasitology is providing an increasingly precise picture of host-parasite interactions: about the molecular signals, pathways, and systems that allow parasites to prosper in their hosts. However, some of the most fundamental actors at the host-parasite interface, although obvious, are chiefly ignored – these are physical and especially mechanical forces, spanning from molecular to tissue scales and beyond. Most parasites thrive in a micro-world, where physical conditions differ dramatically from the ones prevailing in the macro-world that we know. Inertia is negligible, viscosity dominates, and fluid flow can be by turns extraordinarily fast or very slow, laminar or turbulent. Parasites have to cope with these varying physical cues; they have to move and steer, pass through void spaces or swim in extremely crowded environments, full of obstacles and varying degrees of confinement. They have to reversibly attach to surfaces, break through barriers, and penetrate tissues or membranes. They have to tune their power so as not to harm the host, or destroy cells while hiding within them, but they also have to exert sufficient power for egress when the time is right. Parasites can join forces, form swarms and display varying degrees of collective behaviours, many of which are probably independent of chemical signalling, but rather the product of physical cues such as hydrodynamic coupling. Now is the right time to view parasitism from a more physical, micro-engineering point of view. We need to understand the physics of molecular forces generated by motors, bonds, and barriers. We need to understand the build and behaviour of parasites, both as physical entities and highly evolved micromachines. And we need to appreciate the biomechanical cues provided by the parasites' microenvironments. I will introduce this perspective and pinpoint some aspects of physical parasitology, thereby mainly focusing on our work on African trypanosomes as prototypical flagellate microswimmers.

POP-O-02

Narrow escape: How long does it take for a camel to go through the eye of a needle?

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The narrow escape (NE) problem is a common problem in biology and biophysics. It deals with Brownian particles confined to a given domain with reflecting borders and only a small opening where the particles are absorbed. This is reminiscent of variant surface glycoproteins (VSGs) diffusing on the plasma membrane of *Trypanosoma brucei*. In contrast to other eukaryotic cells, *T. brucei* have a sole site for endo- and exocytosis, the flagellar pocket. This small membrane invagination covers only approx. 5% of the surface. The time a particle needs to reach the target by pure Brownian motion, the mean first passage time, has been analytically calculated for several geometries in two and three dimensions. Comparison of the theoretical predicted time with measurements of VSG coat exchange in trypanosomes yields a clear discrepancy in timescales.

We will address the problem in two ways. First, by measuring site-specific VSG diffusion *in vivo* with single-molecule fluorescence microscopy. For this purpose, we recently introduced super-resolution imaging of intrinsically fast moving flagellates based on cyto-compatible hydrogel embedding [Glogger et al. JPD: Appl Phys 2017, Glogger et al. Exp Parasitol 2017]. Second, by challenging the theory of NE experimentally in micro-patterned model membranes. We will vary geometric parameters systematically and test the validity of the theoretical model in a wide phase space. Moreover, we even aim to manipulate the *in vivo* geometry by knockdown of relevant structural proteins.

POP-O-03
An *in silico* model for the African trypanosome

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The African trypanosome is the causative agent of the sleeping sickness and there is tremendous interest in understanding all aspects of how it moves forward and how it interacts with its environment. This includes the blood flow in blood vessels and passing the brain-blood barrier. Therefore, in the past years we have developed an *in silico* model for the African trypanosome, which fairly well captures its swimming motion [1-3]. The trypanosome has a conventional eukaryotic flagellum attached to its body. When a bending wave runs along the flagellum, the whole body deforms and is able to swim in the liquid environment, which me model with a particle-based solver of the Stokes equations called multi-particle collision dynamics.
With the help of the *in silico* model, we are able to demonstrate that the helical attachment of the flagellum optimizes the swimming speed [3], which helps the trypanosome to dispose of antibodies. We also simulate different morphotypes that occur during the parasite's development in the tsetse fly [3]. Finally, we address swimming in confinement and demonstrate that nearby channel walls or obstacles help the trypanosome to move forward.

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POP-O-04
Modeling Malaria Invasion of Red Blood Cells – and Beyond

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The first important step in the blood cell cycle of malaria is the invasion of red blood cells (RBCs) by merozoites. The merozoite has a shape similar to a chicken egg, with a length of about 2 micron. Therefore, its interaction with the RBC membrane shares several features with the endocytosis of other non-spherical nano- and micro-particles into cells [1]. We study wrapping of particles as interplay of the membrane deformation energy and the particle adhesion energy. With the help of numerical energy minimization using triangulated surfaces, we investigate the role of shape and size of the particle as well as of the membrane"s elastic parameters on particle wrapping. For non-spherical

(rod-like, disc-like, cuboidal) particles compared with spherical particles, we find a higher binding affinity to the membrane, partial-wrapped states, and a lower uptake to cells [2].
These theoretical approaches provide the basis for the modeling of merozoite invasion [3]. Merozoites are expected to adhere to a membrane with their least-curved side, because this con- figuration provides the largest adhesion area with the lowest cost in membrane deformation energy. Therefore, the reorientation of the merozoite for apical invasion requires a non-uniform adhesion energy, with an adhesion maximum at the apex. The subsequent wrapping of the merozoite is then be controlled by four energetic contributions: (i) the adhesion strength, (ii) the membrane bending rigidity and its spontaneous curvature, (iii) the lateral tension of the RBC membrane, and (vi) the effective line tension of the tight junction. The importance of active processes, driven by the parasite, are found to be important to cross energy barriers between partial-wrapped and complete-wrapped states [3].
Finally, malaria infection modifies RBC properties, and thereby blood-flow behavior [4,5]. A few of these effects will be discussed, such as the change in blood viscosity.

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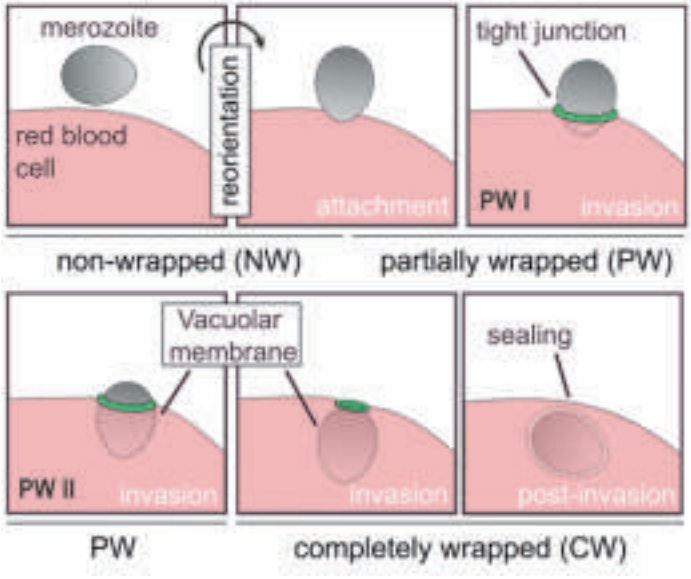
Figure 1



POP-O-05

Modelling cytoadhesion of malaria-infected red blood cells

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In order to avoid clearance by the spleen, malaria-infected red blood cells (iRBCs) develop a system of adhesive knobs that allows them to bind to the vascular endothelium. Combining phase contrast and fluorescent microscopies for iRBCs in flow chambers with endothelial monolayers, we found that trophozoites (intermediate-stage iRBCs) tend to flip due to their biconcave shape while schizonts (late-stage iRBCs) tend to roll due to their almost spherical shape. Using adhesive dynamics simulations for round cells, we establish estimates for the key adhesion parameters of iRBCs, including the on- and off-rates of the adhesive interactions (mainly PfEMP1 and ICAM1), knob density and receptor multiplicity inside the knobs. Using multiparticle collision dynamics (MPCD), we then simulated deformable cells in shear flow and established estimates for the key mechanical parameters, including bending stiffness and shear modulus of the iRBC-envelope. We found that the hemoglobin mutation HbAS, which leads to lower knob density and higher envelope stiffness, also results in a decreased adhesive interaction with the endothelial monolayer when compared to the wildtype HbAA.
These findings help to understand better why the sickle cell disease partially protects against malaria infections and show the power of a quantitative biophysical approach when studying parasitism.

POP-O-06

Understanding force transduction during malaria parasite migration

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Plasmodium sporozoites are the highly motile forms of malaria-causing parasites that are transmitted by Anopheles mosquitoes. Sporozoites form in oocysts at the mosquito midgut and start migrating just before they rupture the oocyst wall. They float in the hemolymph and actively enter salivary glands where they either associate in non-motile groups or slowly disperse within the salivary canals. Upon injection into the skin, the parasites move at high speed and search for blood capillaries. In our work we aim to understand how sporozoite motility is modulated using classic molecular genetic tools. However we also apply quantitative biophysical analysis such as traction force microscopy and laser tweezers to investigate the force generation capacity of wild type and genetically modified sporozoites during their rapid motility. With this multidisciplinary approach we hope to ultimately understand how these parasites manage to move 10 times faster than the fastest mammalian cell.

POP-O-07
The role of actin in apicomplexan: Back on track?

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Actin is one of the most abundant proteins in eukaryotic cells and due to its ability to form polymers is involved in a multitude of critical cellular functions, such as cargo transport, cell motility, membrane dynamics, cytokinesis or gene regulation. In order to define the role and dynamics of F-actin in living cells, the development of imaging techniques using fluorescently labelled F-actin binding proteins was crucial. While for most eukaryotes a well-established toolkit of different F-actin probes is available, in the case of apicomplexan parasites none of the conventional actin probes allowed the reliable localisation of F-actin. Therefore, it has not been possible to study actin *in vivo*, so its physiological role has remained obscure. This has led to functional models which are mutually conflicting, incompatible with actin behaviour from other eukaryotes, and cannot explain actin's importance during basic processes such as host cell invasion, parasite replication and egress.
Here we used Chromobodies to visualise F-actin in the apicomplexan parasites *Toxoplasma gondii* and *Plasmodium falciparum* and followed its localisation and dynamics throughout the asexual life cycle of the parasite. Intriguingly, localisation of F-actin during host cell invasion is not reconcilable with the current models for gliding motility and invasion. Based on kinetic and dynamic measurements we propose a new model where a crucial role of actin lies in nuclear protection and deformability during motility and invasion.

POP-O-08
Understanding the physics of hydatid cysts

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The metacestode larval stages of the tapeworms Echinococcus multilocularis and E. granulosus differ from all other cestode larvae in their cystic morphology and the secretion of an acellular, laminated layer, which is typically several hundred micrometers thick in E. granulosus and much thinner in E. multilocularis. In contrast to E. granulosus, which grows as one solid hydatid cyst, the E. multilocularis metacestode forms a multivesicular tissue that grows infiltrative, like a malignant tumor, into the surrounding host tissue. Previous studies of our group already established that Echinococcus metacestodes are a specialized form of asexually reproducing parasite tissue which results from a posteriorization process of the invading oncosphere larva. We also showed that metacestode growth strictly depends on the proliferation and differentiation of a population of totipotent somatic stem cells which are delivered into the host through the oncosphere larva. So far it is, however, totally unclear how the oncosphere tissue is reorganized towards the cystic metacestode, how the laminated layer is formed through the syncytial germinative layer of the parasite, and how the laminated layer is reorganized in the case of E. multilocularis so that infiltrative growth can be achieved. The physical properties of hydatid cysts are expected to result from dynamic processes initiated at the parasite

cellular layer through stem cells and the formation/reorganization of the laminated layer by the syncytial parasite tegument. In the present project we propose to study these dynamics by means of in vitro cultivation systems for metacestode development, supported by functional genomic methods to manipulate stem cell dynamics and laminated layer production, as well as chemical methods to locally modify laminated layer formation. By interacting with experts in physics we expect added value in deeper understanding of physicochemical aspects of parasite surface dynamics.

Protozoan parasites I

PRO-O-01

Dissecting the expression, localization and function of the *P. falciparum* STEVOR family

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Introduction: The polymorphic STEVOR protein family, encoded by 40 genes within the parasite genome, belongs to the small variant surfaces antigens exposed on the surface of infected erythrocytes (iE) during the intra-erythrocytic cycle of *Plasmodium falciparum*. Members have been localized at the host cell membrane, where they mediate the binding of iE to uninfected erythrocytes in terms of rosetting and contribute to the stiffness of iE. They were also found at the apical organelles as well as at the plasma membrane of merozoites suggesting additional role during host cell invasion.
Objectives: We investigate the role of different STEVOR expression pattern for their different localizations and putative functions.
Materials & Methods: The expression of *stevor* during intra-erythrocytic cycle of the parasite has been determined using RNA sequencing and qPCR with subsequent modeling of the profiles. Expression profiles were confirmed on protein level by Western blot using specific antibodies against selected STEVOR variants. Furthermore, the localization of selected STEVOR was investigated using antibodies and transgenic parasites lines in immunofluorescence and live microscopy analysis. Finally, the function of these STEVOR variants was assessed by growth inhibition assays.
Results: STEVOR variants show individual gene expression and localization patterns during the intra-erythrocytic cycle. Strikingly, many *stevor* genes not only show an expression peak during the trophozoite stage but they also exhibit a second increase of expression in the late parasite stages like schizonts or merozoites. Moreover, specific antisera against a particular STEVOR variant showed a significant inhibition of parasite growth supporting their role during invasion of new host cells.
Conclusion: Our results support the hypothesis that the different functions of STEVOR proteins are the consequence of their individual gene expression and localization patterns.

PRO-O-02

Towards understanding epigenetic gene regulation of differentiating male and female *P. falciparum* gametocytes

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P. falciparum causes the most severe form of human malaria. The symptoms of malaria are associated with the invasion of erythrocytes, in which the parasites replicate asexually. Transmission to the mosquito vector is mediated by a small proportion of parasites which differentiate into male and female gametocytes. Mature gametocytes are morphologically and metabolically very distinct from asexual parasites and from each other, reflecting their fundamentally different biological roles. *P. falciparum* gametocytes are characterized by a very long maturation period in the human host, the significance of which is not well understood. To gain insight into sex-specific transcriptional changes during the course of gametocyte differentiation, we separated immature and mature male and female gametocytes using a flow cytometric approach. Directional RNAseq revealed that male and female transcriptomes differ significantly from each other from an early time point during gametocyte maturation onwards. Differentiation processes in eukaryotes are associated with changes in the epigenetic landscape which direct the stage-specific regulation of genes. To determine how chromatin modifications correlate with gene transcription in developing gametocytes, we performed genome wide chromatin immunoprecipitation (ChIPseq) of several histones and histone modifications and compared the profiles to asexual parasites. The data revealed that Histone 3 lysine 27 acetylation (H3K27ac) enrichment at transcriptional start sites correlates with gene expression in both asexual and sexual parasites. Unexpectedly, we found that histone 3 lysine 4 tri-methylation (H3K4me3), which is associated with intergenic regions and shows a strong enrichment at the start codon of highly transcribed genes in asexual parasites, is widely redistributed to coding sequences in gametocytes. The level of enrichment in the coding sequences correlates with gene activity in gametocytes, suggesting that H3K4me3 may serve as a marker of cellular identity in differentiating *P. falciparum* parasites. Together, our data provide unique and novel insight into the mechanisms underlying *P. falciparum* gametocyte differentiation.

PRO-O-03

Complement factor H-related protein 1 impairs factor H acquisition during complement evasion the by malaria parasite *Plasmodium falciparum*.

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Introduction: The complement system is an essential component of the innate immune response and the first defense line against invading pathogens like the malaria parasite *Plasmodium falciparum*. We have previously shown that the blood and transmission stages of *P. falciparum* bind the complement

regulator factor H (FH) to inactivate C3b and thus evade lysis by the membrane attack complex. The regulatory activity of FH is modulated by the members of the factor H-related (FHR) protein family, including FHR-1. FHR-1 is composed of five short consensus repeat domains with variable homology to similar domains of FH. While FHR-1 also binds C3b, it lacks the regulatory activity to achieve C3b inactivation.

Objective: The objective of the study was to determine, if and how FHR-1 might modulate the regulatory activity of FH during complement evasion by the *P. falciparum* blood stages and thus affect survival of the malaria parasite.

Materials & Methods: We employed cell-based binding assays, enzyme-linked immunosorbent assays, and indirect immunofluorescence assays, as well as growth and invasion assays using purified schizonts and merozoites incubated either with human normal or FHR-1 deficiency serum.

Results & Conclusion: We demonstrate that both schizonts and merozoites bind FHR-1 in addition to FH, while FHR-1 is not found on the surface of non-infected red blood cells. Binding of FHR-1 results in reduced acquisition of FH by the parasite, while FHR-1 deficiency causes increased FH binding. In consequence, FHR-1 binding by the parasite leads to a reduced overall parasitaemia of the blood cultures, while sera from people with FHR-1 deficiency favors parasite growth and increased red blood cell infection rates. We hypothesize that FHR-1 acts as a decoy to counteract FH-mediated microbial complement evasion.

PRO-O-04

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

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Question: Severe anemia represents one of the major complications in malaria and affects primarily young children. Red blood cell destruction by the causative agent of malaria, intra-erythrocytic *Plasmodium* parasites, is the main origin of anemia, but clinical data suggests that additional factors add to the pathology. The underlying mechanisms that explain the disproportional loss of erythrocytes when compared to the proportion of infected cells remain to be elucidated. One attractive hypothesis is coating and immune recognition leading to phagocytosis of uninfected erythrocytes with *Plasmodium* antigens, which are released into the blood stream upon parasite egress and during intra-erythrocytic replication through microvesicles. In this study, we investigated microvesicle-mediated transfer of parasitic material to uninfected erythrocytes and its contribution to phagocytic destruction.

Method: We purified microvesicles from *Plasmodium falciparum* cultures and characterized the protein content by mass spectrometry.

Results: We show that a signature microvesicle protein, ring-infected erythrocyte surface antigen (RESA), can be found on the surface of non-infected erythrocytes. Transwell co-culture experiments further substantiate our notion that coating of normal erythrocytes by parasite antigens occurs through microvesicle transfer. We finally tested whether this mechanism primes uninfected cells for phagocytosis *in vitro* by macrophages.

Conclusion: Our findings show that microvesicles derived from *Plasmodium*-infected erythrocytes can deposit parasite antigens on uninfected red blood cells. Whether this mechanism contributes to the pathogenesis of malaria-related anemia can now be examined in clinical studies.

PRO-O-05
***P. falciparum* glycans in adaptive immune recognition and their vaccine potential**

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Glycosylphosphatidylinositol (GPI)-specific antibodies are postulated to be protective against severe malaria pathogenesis. Their effector function and epitope specificity are not fully understood.
We will discuss the rationale for a hypothetical minimum protective epitope that directs a subset of GPI-specific antibodies toward the glycan portion of the full GPI, and outline our approach for characterising the effector function of these antibodies.
Their induction through vaccination, their effect on animal challenge models, and the implications for vaccine synthesis and formulation will be evaluated.

PRO-O-06
Epidemiological aspects of Phlebotomine sand fly species, Leishmania vectors, from Old and New World: Alentejo region, Southern Portugal, and Volta Redonda Municipality, Brazil, 2016/2017

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Portugal and Brazil are human and canine visceral leishmaniasis neglected regions: *Leishmania infantum* is the pathogen, dogs the principal reservoirs, humans the hosts and sand fly species the vectors. The Mediterranean basin leishmaniasis epidemiology is changing, and the sand fly species in Alentejo, have not been studied for more than a decade. In Volta Redonda, Brazil, where the first human and canine leishmaniasis cases were reported in 2014, the sand fly species were not systemised studied and other vectorial aspects are unknown.
We aimed to explore the sand fly fauna, to determine the abundance, density, molecular *L. infantum* infection and higher transmission season risk.
Specimens were captured monthly by CDC traps followed by morphological identification. To evaluate the natural Leishmania infection, molecular diagnosis was performed: PCR multiplex; specific primers.
In Portugal, in July, 4 sand fly species were identified and *Phlebotomus perniciosus* and *P. sergenti* were the most abundant species. In the same month, the highest phlebotomine density and Leishmania vectorial risk was observed. For the first time, *Sergentomyia minuta* tested positive for *L. infantum* infection. In Brazil, *Lutzomyia longipalpis* was the most abundant specie and February and

March presented the highest densities and vectorial transmission risk. *L. longipalpis* and *Evandromyia sallesi* tested positive for *L. infantum*.
Further surveillance studies on vectors should be promoted and, with the ambient and climatic changes, strategies to control zoonotic vector borne diseases have to be rethought in the Old and New World.

PRO-O-07

***Toxoplasma gondii* infections in chickens - Performance of various antibody detection techniques in serum and meat juice relative to bioassay and DNA detection methods**

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Introduction: Chickens, especially if ranged free, are highly susceptible for infection with *Toxoplasma gondii* and represent important reservoir animals.
Objective: The aim of the present study was to determine the suitability of commonly used antibody detection methods, i.e. the modified agglutination test (MAT), the indirect immunofluorescence test (IFAT) and enzyme-linked immunosorbent assay (ELISA) to detect *T. gondii*-infected chickens. Serum and various sources of meat juice were tested for antibodies to *T. gondii* to determine the suitability of these analytes in combination with various antibody detection methods.
Material & Methods: The infection status of experimentally and naturally exposed chickens was determined by Magnetic-Capture PCR. Naturally exposed chickens were in addition examined by mouse bioassay and conventional real-time PCR on acidic pepsin digests.
Results: In experimentally and in naturally infected chickens, brain and heart tissues harbored an at least 100-times higher parasite concentration than breast, thigh or drumstick musculature. Under experimental conditions, the agreement between MC-PCR and the serological techniques revealed Kappa values of 0.67 (ELISA), 0.58 (IFAT) and 0.70 (MAT). Under field conditions, examinations of sera by ELISA, IFAT and MAT showed a good performance in identifying mouse bioassay-, Magnetic-Capture PCR-, or conventional real-time PCR-positive chickens as illustrated by Kappa values of 0.74, 0.70 and 0.65, respectively. The examination of meat juice samples from breast, drumstick or heart musculature revealed similar or even better results in IFAT and ELISA than the examination with serum. The meat juice results in MAT were less consistent due to false positive reactions.
Conclusion: ELISA, IFAT and MAT performed well in identifying mouse bioassay-, MC-PCR- or conventional real-time PCR-positive chickens, regardless if blood serum or meat juice from different muscle tissues were applied.

PRO-O-08
Use of CRISPR/cas 9 to establish transgenic *Cryptosporidium parvum* strain in vitro

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Cryptosporidium parvum is a common and worldwide distributed intestinal protozoan parasite which causes diarrhea of variable severity in young animals, particularly ruminants, and children. In immunocompromised or malnourished individuals devastating and even lethal disease may occur. Despite the long known impact of this disease in human and veterinary medicine ("one health"), sufficiently effective drugs for treatment or prevention are lacking. Calcium-dependent protein kinases proteins (CDPKs) are believed to play critical roles in apicomplexan protozoa and appear to essentially contribute to accomplishment of the parasitic life-cycle. It has been demonstrated experimentally, that CDPK1 inhibitors ("bumped kinase inhibitors", BKI) hamper parasite development in vitro and reduce parasite reproduction in the natural host of *C. parvum*. Further functional studies on CDPKs are placed in hope to lead promising solutions to the disease. Therefore, we are constructing the CDPK1 knock-out strain which could be utilized for the aim. In the present study, CRISPR/cas 9 technology was used to replace genomic CDPK1 with Nluc-NeoR cassette which offers the parasites paromomycin resistance. Transfection was done by using highly efficient electroporation-free cell-penetrating peptide that requires as little as only 1 µg of plasmid DNA. Moreover, the COLO-680N (Human oesophageal squamous-cell carcinoma) cell line was applied instead of using animal models for propagation. This cell has been proved capable of propagating *C.parvum* without intensive cares.

PRO-O-09
Use it or lose it: function and functionality of mitochondrial DNA in the sleeping sickness parasite *Trypanosoma brucei*

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Question: Trypanosomes depend on correct function of their single mitochondrion; a number of anti-trypanosomatid drugs interfere with replication of the parasite's mtDNA, the kinetoplast. Function of the *T. brucei* mitochondrion is strictly regulated: in the procyclic stage in the tsetse fly midgut proline fuels mitochondrial ATP production; in the proliferative slender bloodstream form in the mammalian host cytosolic ATP production via glycolysis is the main (and perhaps only) source of energy. Mitochondrial gene expression is essential in slender forms, but single point mutations in the F_1F_O-ATPase γ subunit (e.g. L262P) permit viability of akinetoplastic (AK) forms and result in multidrug resistance. Whether these mutations also permit survival of AK insect forms, and thus enable transmission of drug resistant parasites, is unknown.

Methods: We have generated AK mutants in the genetic background of a transmission competent *T. brucei* strain and investigated the consequences of F_1F_O-ATPase mutation and mtDNA loss *in vitro* and *in vivo*. We have also generated the first complete assemblies of the kinetoplast genome, before and after mating in the tsetse fly.

Results:

- AK slender forms can differentiate into stumpy forms, the transmission stage; however, AK stumpy forms have a reduced life span and lack a mitochondrial membrane potential
- AK stumpy forms cannot infect tsetse flies
- Heterozygous L262P mutants (which are as drug resistant as AK parasites) can complete the entire life cycle - Homozygous L262P mutants have impaired F_1F_O-ATP synthase function; they establish a midgut infection but not a salivary gland infection, indicating that efficient oxidative phosphorylation is required to power migration in the tsetse fly.
- Kinetoplast complexity is lost during the bloodstream stage; mating in the tsetse fly restores complexity, with progeny inheriting "minicircles" from both parents in equal parts.

PRO-O-10

The novel telomere-associated protein TelAP1 links stage-specific telomere complexes with developmental expression site silencing in African trypanosomes

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Mammalian telomere-binding proteins are organized in the shelterin complex, which is indispensable for telomere functions. In *Trypanosoma brucei*, the causative agent of African trypanosomiasis (sleeping sickness), three homologous proteins of the shelterin complex have been identified so far - TbTRF, TbRAP1, TbTIF2. These proteins are important for antigenic variation, a mechanism trypanosomes use to evade the host immune response by periodically switching their variant surface glycoprotein (VSG) coat. Key mediators of this process are 15 specialized subtelomeric transcription units named expression sites (ES). Each ES contains one VSG gene. At any given time, only one ES is transcriptionally active in the mammalian infectious bloodstream form (BSF) of the parasite. All 15 ES are inactive in the insect vector stage procyclic form (PCF). Previous reports have shown that TbTRF, TbRAP1, and TbTIF2 are involved in ES regulation. However, the precise nature of their contribution remains unclear. To understand this mechanism, it is first essential to determine the complete composition of the trypanosome shelterin complex.

We used two independent approaches to find novel components of the shelterin complex in *T. brucei* – (i) a pull-down assay with telomeric repeat oligonucleotides; (ii) a co-immunoprecipitation to find novel TbTRF-interacting proteins. We isolated 17 putative telomere-associated proteins including known telomeric proteins such as TbTIF2. Initial characterization of the novel telomere- associated protein 1 (TelAP1) hints to the formation of stage-specific telomere complexes. TelAP1 was verified as a shelterin component by indirect immunofluorescence analysis and reciprocal co-immunoprecipitation. Mass spectrometry and immunoblot analysis showed a 4-fold upregulation of TelAP1 in BSF compared to PCF. Interestingly, VSG silencing was faster in TelAP1 -depleted cells during differentiation from BSF to PCF.

PRO-O-11
Viral discovery and diversity in trypanosomatids with a focus on relatives of the human parasite *Leishmania*

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Knowledge of viral diversity is expanding greatly but many lineages remain underexplored. We surveyed RNA viruses in 52 cultured monoxenous relatives of the human parasite *Leishmania* (*Crithidia* and *Leptomonas*), as well as plant-infecting *Phytomonas*. *Leptomonas pyrrhocoris* was a hotbed for viral discovery, carrying a new virus (*Leptomonas pyrrhocoris* ostravirus 1) with a highly divergent RNA dependent RNA polymerase missed by conventional BLAST searches, an emergent clade of tombus-like viruses, and an example of viral endogenization. A deep branching clade of trypanosomatid narnaviruses was found, notable as *Leptomonas seymouri* bearing narna-like virus 1 (LepseyNLV1) have been reported in cultures recovered from patients with visceral leishmaniasis. A deep branching trypanosomatid viral lineage showing strong affinities to bunyaviruses was termed "Leishbunyavirus" (LBV), and judged sufficiently distinct to warrant assignment within a new proposed family termed "Leishbunyaviridae". Numerous relatives of trypanosomatid viruses were found in insect meta-transcriptomic surveys, which likely arise from trypanosomatid microbiota. Despite extensive sampling we found no relatives of the totivirus *Leishmaniavirus* (LRV1/2), implying that it was acquired at about the same time the *Leishmania* became able to parasitize vertebrates. As the new viruses were found in over a quarter of isolates tested, more are likely to be found in the >600 unsurveyed trypanosomatid species. Viral loss was occasionally observed in culture, providing potentially isogenic virus-free lines enabling studies probing the biological role of trypanosomatid viruses. These data shed important insights on the emergence of viruses within an important trypanosomatid clade relevant to human disease.

PRO-O-12
Trans-acting GC-rich non-coding RNA at var expression site modulates gene counting in malaria parasite

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Monoallelic expression of the var multigene family enables immune evasion of the malaria parasite Plasmodium falciparum in its human host. At a given time only a single member of the 60-member var gene family is expressed at a discrete perinuclear region called the 'var expression site'. However, the mechanism of var gene counting remains ill-defined. We hypothesize that activation factors associating specifically with the expression site play a key role in this process. Here, we investigate the role of a GC-rich non-coding RNA (ncRNA) gene family composed of 15 highly homologous members. GC-rich genes are positioned adjacent to var genes in chromosome-central gene clusters but are absent near subtelomeric var genes. Fluorescence in situ hybridization demonstrates that GC-rich

ncRNA localizes to the perinuclear expression site of central and subtelomeric var genes in trans. Importantly, overexpression of distinct GC-rich ncRNA members disrupts the gene counting process at the single cell level and results in activation of a specific subset of var genes in distinct clones. We identify the first trans-acting factor targeted to the elusive perinuclear var expression site and open up new avenues to investigate ncRNA function in antigenic variation of malaria and other protozoan pathogens.

PRO-O-13
An unusual prohibitin regulates malaria parasite mitochondrial membrane potential

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Proteins of the stomatin/prohibitin/flotillin/HflK/C (SPFH) family are membrane-anchored and perform diverse cellular functions in different organelles. Here, we investigate the SPFH proteins of the murine malaria model parasite Plasmodium berghei, the conserved prohibitin 1, prohibitin 2, stomatin-like protein, and an unusual prohibitin-like protein (PHBL). The SPFH proteins localize to the parasite mitochondrion, and systematic gene targeting suggests essential functions for the three conserved SPFH proteins during blood infection. In contrast, PHBL was successfully ablated, but its absence impaired asexual parasite propagation and virulence. Strikingly, PHBL-deficient parasites fail to colonize the Anopheles vector due to a specific arrest during ookinete development in vivo. We show that this arrest correlates with the depolarization of the mitochondrial membrane potential ($\Delta\Psi$mt). Our results underline the importance of SPFH proteins in the regulation of core mitochondrial functions and suggest that fine-tuning of $\Delta\Psi$mt in malarial parasites is critical for the colonization of the definitive host.

PRO-O-14
Profile of cholesterol-related sterols and impact of selected oxysterols in *Eimeria bovis* macromeront formation in bovine host endothelial cells

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Introduction: During first merogony *Eimeria bovis* forms large macromeronts in host endothelial cells. Especially for offspring membrane biosynthesis, large amounts of cholesterol are needed for a

successful replication process. Given that apicomplexan parasites are generally considered deficient in cholesterol biosynthesis, it has to scavenge cholesterol from its host cell.
Objective: We here analysed the impact of intracellular *E. bovis* infection on host cellular cholesterol-related sterol profile by measuring precursors of endogenous cholesterol synthesis, phytosterols as indicators of sterol-uptake and oxysterols as indicators of cholesterol conversion. Furthermore, we estimated the effect of oxysterol treatments on parasite development.
Material and Methods: *E. bovis* macromeronts were cultured in bovine endothelial cells under normal and merozoite I-boosting conditions. At 14 days p. i., infected and non-infected host cells were analysed via GC-MS for content of cholesterol precursors, i. e. phytosterols and oxysterols. Furthermore, *E. bovis*-infected BUVEC were treated with oxysterols (25-OHC, 27-OHC, 7 ketocholesterol) from 10 days p. i. onwards and the effect of this treatment on macromeront development was estimated.
Results: The current data show a simultaneous induction of host cellular endogenous cholesterol synthesis and exogenous sterol up-take in the case of optimal merozoite I production. In addition, enhanced synthesis of ring-modified oxysterols indicate considerable cell stress driven by *E. bovis* infection. Furthermore, treatments with 25-OHC, 27-OHC and 7 ketocholesterol, significantly inhibit macromeront development and merozoite I production.
Conclusion: Current data indicate a dramatic change of cholesterol-related sterol profile of *E. bovis*-infected host endothelial cells in conditions of optimal parasite replication reflecting huge demand of this parasite for cholesterol and its versatility in the acquisition of cholesterol sources.

Symposium • Emerging vector-born parasitic zoonoses

SEP-O-01

Veterinary relevance and potential for spread of dirofilariosis

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Introduction: *Dirofilaria immitis* and *Dirofilaria repens* are two mosquito-borne nematodes that infect primarily dogs. Both are zoonotic and human infection with *D. repens* is currently considered an emerging problem in many areas of Europe.
Veterinary relevance of dirofilarosis: Canine Heartworm disease (CHWD), caused by *D. immitis*, is a chronic disease of the pulmonary arteries and lungs. Adult parasites live in the pulmonary arteries and consequent endoartheritis and perivascular inflammation lead to a rise in pulmonary pressure and right-side congestive heart failure. Most dogs with subcutaneous dirofilariosis, caused by *D. repens*, remain asymptomatic. When present, the primary clinical sign is the presence of skin nodules, located in different anatomical sites. Sporadic reports of erythema, papules, alopecia and pruritus have also been described in dogs with natural *D. repens* infection.
Potential for spread of dirofilariosis: The movement of infected dogs, the presence/introduction of competent mosquito vectors and climate changes that allow the development of infective larvae and the survival of mosquitos for longer periods of the year, are all currently contributing to the spread of *Dirofilaria* spp. infection and disease from the traditional endemic areas of southern Europe towards more northern areas. Beginning approximately 10 years ago, an increasing number of autochthonous cases of *D. repens* infection has been reported in Germany, including the areas of Baden-Wurttemberg and Brandenburg. In these same areas, molecular surveys of captured mosquitos indicate that both *D.*

immitis and *D. repens* are present. Recent climate-based mapping has confirmed that native cycles of parasite transmission are likely established in Germany.
Conclusions: Chemoprophylaxis of *Dirofilaria* spp. infection in dogs is the hallmark of controlling further spread of the parasite and of safeguarding human and animal health in areas at risk.

SEP-O-02
emerging Human dirofilariasis as a medical problem

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Human dirofilariasis (HD) is an emerging disease in Europe. More than 4000 cases of HD were reported from the former USSR states during the last two decades (mostly from Ukraine, Russia and Belarus).
Based on 323 patients records we have analyzed HD clinical presentation. A seroepidemiology survey involved 318 healthy people. Molecular study included 49 HD surgically excised worms. Our results showed that HD mostly affects 20 - 60 years old patients (41%), women are much more often affected than men (71%). Anatomical location in an eyeball conjunctiva and eye surrounding area (39%) followed by head and neck (25%), then limbs (22%) and trunk (11%), and rarely men genitalia (3%) and women breast (2%) are the sites of HD parasite location. DNA amplification of specific 12S rRNA subunit of the excised parasites followed by sequences based phylogenetic analysis revealed that 14% of HD ocular cases were caused by *Dirofilaria immitis*. Seroepidemiological study showed that 10% of healthy population in south-western Russia are seropositive. The majority of HD patients had no symptoms (excluding eyes and genitals location). HD is not well known to medical doctors especially in the countries where the disease is rear or presented as imported cases. The most of clinical pre-surgery diagnoses were benign or malignant tumors. At the same time global educational and notification system introduced from 1997 in Ukraine resulted in as high as 75% of "HD suspicion" primary diagnosis there. Based on our focused study we state that the only way to suspect a well-grounded pre-surgery diagnosis is an ultrasound examination including color Doppler charting that allows finding some well-defined characteristics that are specific for HD. The emergence of HD in Europe, the established role of *D. immitis* in some of HD ocular cases, the high seroprevalence in healthy population should stimulate interdisciplinary and international research of HD.

SEP-O-03
Clinical management of canine leishmaniosis: towards a One Health approach

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Canine leishmaniosis due to *Leishmania infantum* is endemic in southern Europe (Otranto et al., 2017; Maia and Cardoso, 2015) and prevalences of infection (estimated by serologic and molecular diagnosis) can be as high as 60% in exposed populations, including at least 2.5 million seropositive dogs. Outside of this area, many imported cases of canine leishmaniosis have been reported. Thus, in the absence of efficient vectors in non-endemic regions, other sources of infection are possible including blood transfusions, the use of products derived from infected donors, and vertical or venereal transmission. So far, reports of direct transmission have been scarce.
When we speak of dogs and leishmaniosis we should insist that infection does not equate to disease as there is a high prevalence of subclinical infections. This means that in an endemic area, there is a high prevalence of infected dogs (estimated at close to 60% by molecular methods) compared with the number of dogs that are really sick from leishmaniosis (some 5-10%). A dog infected with *Leishmania* is considered healthy if, despite no clinical signs or laboratory abnormalities, we can demonstrate the presence of the parasite (despite low antibody levels) using highly sensitive diagnostic tests (polymerase chain reaction; PCR). In contrast, when the presence of the parasite can be similarly detected yet clinical signs and/or laboratory abnormalities compatible with leishmaniosis are present, the dog is considered sick. In these dogs, antibody levels against the parasite are usually at a medium to high level.
An infected dog may become sick during its life according to the immune response and/or parasite challenge. In those dogs, progression to disease is associated with reduced T-cell mediated immunity and a marked humoral response thought a mixed a mixed Th1 and Th2 cytokine response was detected in *L. infantum* naturally -infected dogs.
Lastly, any concomitant disease or stress may prompt a change in the immune response and some infected dogs may become sick. It seems that this fact is the most likely usually occurs in non-endemic areas where dogs previously travelling to endemic areas have acquired the infection.
The diagnosis of canine leishmaniosis is complex and multiple strategies need to be used. It is recommended according to the clinical signs detected and laboratory results, to combine the clinical diagnosis with specific techniques such as quantitative serology (immunofluorescence antibody test, IFAT, or the enzyme-linked immunosorbent assay, ELISA) to analyze the humoral response (considering elevated antibody levels as diagnostic in dogs with compatible clinical signs and/or clinicopathological abnormalities). In addition, along with evidence of the parasite in target organs (bone marrow, lymph nodes, skin, spleen), molecular biology methods exist to detect specific DNA through cytology and/or biopsy (conventional PCR, nested PCR, qPCR). Other less invasive samples that may be used are blood, oral/conjunctiva mucosal swabs hair, or urine. However, it has been shown that molecular tests on these samples show reduced sensitivity. Though they are not recommended for stablish a clear diagnosis. Other diagnostic methods are of limited clinical use and require special equipment typical of research institutions. Analysis of the cell immune response via a skin test or delayed hypersensitivity test and lymphocyte proliferation assay; culture in specific media and xenodiagnosis, both by isolating *L. infantum* in laboratory animals or in phlebotomine sand flies

feeding on a suspect host; and histopathology and immunohistochemistry on biopsies of target organs.

Related clinical diagnosis, both the broad range of clinical signs present in sick dogs and the different pathogenic mechanisms and individual immune responses mean we cannot attribute a set of specific signs to this disease. The most frequent signs encountered are cutaneous lesions accompanied by peripheral lymphadenomegaly, which occurs in 50% of cases, along with asthenia, weight loss and muscle atrophy. Progressively, if the dog fails to respond to treatment or in severe infection, a clinical picture of worse prognosis associated with immunocomplex deposition arises: signs of polyuria/polydipsia associated with immunomediated glomerulonephritis, ocular lesions, hepatosplenomegaly, polyarthritis with intermittent lameness, and vasculitis. These four clinical syndromes are of the worse prognosis. In endemic areas, only one of the above described signs is sufficient to suspect canine leishmaniosis.

Even in early disease stages, kidney abnormalities may be clinically reversible though they are considered of poor prognosis as they are usually the main cause of death in the leishmaniosis patient.

In a dog in which leishmaniosis is suspected, the first step is to perform a complete blood count, renal and hepatic biochemical profiles, electrophoretogram of serum proteins and urinalysis. Despite the absence of specific clinicopathological abnormalities, most frequent alterations are non-regenerative anaemia (associated with kidney disease), leucopenia/leucocytosis, thrombocytopenia, hyperproteinemia, hypoalbuminemia, hypergammaglobulinemia and a reduced albumin/globulin ratio. Besides the biochemical profile, it may be possible to observe azotemia, and higher hepatic enzymes (alanine aminotransferase and alkaline phosphatase mainly). Finally, in the urinalysis of dogs with renal disease, we can observe haematuria, proteinuria and isostenuria Urinalysis is therefore a mandatory test for the early diagnosis of glomerular involvement.

It should be underscored that infected but clinically healthy dogs do not require immediate treatment against the parasite. These animals should be monitored for the early detection of possible clinical signs and/or laboratory abnormalities, and increase of antibodies titration suggesting the presence of disease. The use of unnecessary treatments could upset the balance of immunocompetence that these dogs have. On the contrary, sick dogs should be started on therapy as soon as possible for a better response to treatment.

For some years, the success of antimonials has been dependent on its combination with allopurinol as it has been shown that these two active components act synergistically. This means a greater efficacy when lower doses are given over shorter time periods, leading to an increased percentage of cure and to later recurrences. The regimens described as most effective in published guidelines for clinical veterinarians are pentavalent antimonials, as the option of choice, followed by miltefosine. While allopurinol is sometimes given as monotherapy, the efficacy of this drug has been questioned, especially since this compound does not exert a leishmanicidal effect determining that the parasite is never cleared from the host and can be detected at any moment.

An adequate treatment regimen needs to be identified based on the findings of clinical assessment of the dog in which disease is classified according to the severity of clinical signs and/or laboratory abnormalities.

In addition to leishmanicidal agents, immunomodulators such as domperidone, a gastrointestinal prokinetic drug and prolactin stimulator, are given to try triggering a cellular immune response. More recently, it has been reported that the administration of certain nucleotides in combination with the leishmanicidal treatments leads to good clinical improvement.

Most treated dogs experience clinical improvement during the first weeks of treatment though some require a little more time. Parasite identification in lymph node or bone marrow cytology samples, normally associated with progressively increasing antibody levels, is clear evidence of infection

progression towards disease. Some infected healthy dogs develop the disease over a period varying from weeks to years, but most remain without clinical signs or laboratory abnormalities throughout their lives. In general, the prognosis depends on the severity of clinicopathological signs, especially those related to renal function and the individual response to treatment. For this reason, it is recommended that dogs with leishmaniosis and renal disease are assessed based on international IRIS (International Interest Renal Society) guidelines.

Finally, on regards prevention, the only measure that has shown adequate behaviour in mitigating this infection is the regular topical use of repellent insecticides (pyrethroids) in the form of collars, spot-ons and sprays. In areas where leishmaniosis is endemic, we recommend the use of topical pyrethroids (deltamethrin, permethrin, flumethrin).

The preventive efficacy (mass prevention) of the use of pyrethroids only in dogs as shown by different studies is close to 100% in terms of humans or dogs living near such protected dogs.

Several vaccine types have been tested in dogs: inactivated, purified antigens, recombinant antigens, or DNA vaccines. Vaccines based on recombinant antigens or their excretion-secretion products have so far provided the best results. The three currently licensed anti-*Leishmania* vaccines for veterinary use include a recombinant single-protein antigen (available in Brazil), and a secreted/excreted antigen and a recombinant polypeptide antigen (both available in Europe). These vaccines provide 12 months of protection with the clear indication (accepted by the respective medicines agencies) to reduce the risk of developing an active infection and clinical disease after contact with *L. infantum*. Therefore, to achieve maximum protection as the efficacy of available vaccines does not reach 70%, and does not avoid the infection, these should be always used in combination with repellents against sand flies. Today this is considered the best strategy to control canine leishmaniosis. Other applicable measures for the control of canine leishmaniosis includes: keeping dogs inside the home from dusk until dawn during the risk season (May to November in most southern European endemic areas). This is when female phlebotomine sand flies are most active after leaving their resting places to feed. This measure has been shown to also reduce the incidence of infection in dogs. A second measure is to reduce the microhabitats of the arthropod vectors so that eggs are laid in so-called "breeding sites" even this is a very difficult achievement even cleaning organic matter areas, in the surrounding of homes and places where dogs are living, should be always considered.

Furthermore, dogs from non-endemic areas travelling to endemic ones should be protected with repellents before travel and should be testing for *L. infantum* infection (6 months after last travel, by quantitative serology) (Miró et al., 2017).

As a conclusion, it has been well established that special veterinary attention in the last decades focussed on the early detection of infected dogs and the implementation of adequate prevention measures has considerably reduced their infection capacity. Such controlled dogs now pose a much reduced public health risk.

Hence, public health and animal health specialists had to work side by side to design appropriate control measures to fit the new epidemiological situation, as well as provide communication routes and information for health professionals and the general public under the One Health concept.

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SEP-O-04
Emerging human leishmaniosis in Europe: The need of One Health approach

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Human leishmaniosis is considered a neglected tropical disease (NTD), it is present in close to 100 countries and there are more than 1.3 million new cases detected each year including both clinical forms of leishmaniosis, visceral (VL) and cutaneous (CL) (VL 0.3 million, CL 1 million). For the European region, annual incidences of VL and CL reported by the World Health Organization (WHO) run at 1100 to 1900 cases, and 10 000 to 17 000 cases, respectively. For VL, Georgia, Spain, Albania, Italy, Turkey, Tajikistan and Azerbaijan are the most affected countries while CL has been mostly detected in Turkey, Israel, Tajikistan, Turkmenistan and Uzbekistan. In Europe, VL is caused exclusively by *L. infantum* and CL mostly by *L. infantum* (cutaneous and visceral strains differ genetically). Reports exist of rare cases of CL due to *L. tropica* and *L. donovani* in Greece and Cyprus respectively.
In the recent epidemic outbreak of human leishmaniosis due to *L. infantum* in southeast Madrid, the central government of Spain acted by commissioning scientists of multiple fields (physicians, veterinarians, entomologists, epidemiologists, environmental health experts) to discover the cause of the dramatic increase in cases of human leishmaniosis, inform the public and establish adequate preventive measures to deal with the outbreak. Hence, public health and animal health specialists have to work side by side to design appropriate control measures to fit the new epidemiological situation as well as provide communication routes and information for health professionals and the general public. At this stage in the One Health concept, we strive to reach a high degree of understanding and learn from the "two medicines" converting them into one.
Together with Dr. Miró talk, the objective is to compare the current clinical management of leishmaniosis from two perspectives: that of a veterinarian specialized in infectious and parasitic diseases, and that of a physician specialized in infectious tropical diseases.

Vectors, entomology and acarology I

VEA-O-01
Culex torrentium* mosquitoes from Germany are negative for *Wolbachia

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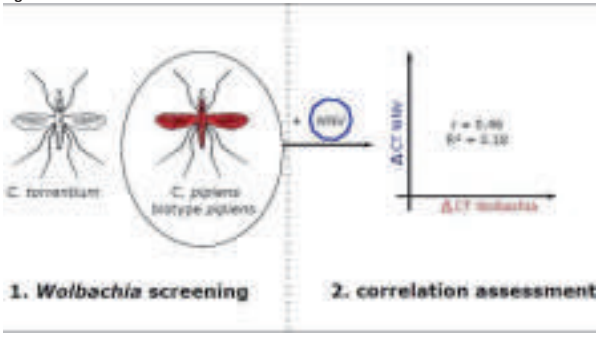
Wolbachia infects a wide range of arthropods, including several mosquito species. The bacterium is known to induce a plethora of phenotypes in its host, examples being the reproductive phenotype cytoplasmic incompatibility or resistance against infection with arboviruses. The latter is especially

relevant when assessing the vector competence of mosquito species for emerging arboviruses. Thus, knowledge of *Wolbachia* infection status is important for the assessment of vector competence.
To facilitate *Wolbachia* screenings in mosquito populations, we developed a qPCR assay that enables high-throughput analysis of mosquito samples. Using this assay, the *Wolbachia* infection status of the two most common *Culex* mosquito species in Germany – *Culex pipiens* biotype *pipiens* and *Culex torrentium* was assessed. About 93 % of all tested *Culex pipiens* biotype *pipiens* individuals were positive for *Wolbachia*, whereas none of the *Culex torrentium* samples were found to be infected. Furthermore, we explored other applications of the qPCR assay by assessing a potential link between the level of *Wolbachia* and WNV infection in German *Culex pipiens* biotype *pipiens* mosquitoes. We found no relationship between the two variables, indicating that a *Wolbachia*-induced antiviral phenotype in this mosquito population is not exclusively due to the general level of bacterial infection

Figure 1



VEA-O-02
Mosquito-based survey of filarial nematodes circulating in Germany

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Xenomonitoring – the screening for pathogens in haematophagous arthropods – can be used to display the occurrence of vertebrate blood parasites, such as filarial nematodes, in a given area. Filarial infections are common and widespread in Europe with some of them being very species-specific and occurring only in animals and others having a remarkable zoonotic potential. It is assumed that many filarial species circulating in Europe are not yet described or are completely unknown. This report updates the findings of filarial nematodes in wild-caught mosquitoes and assesses these in relation to mosquito vector competence for arboviruses. Since 2011, a subset of mosquitoes collected and identified in the framework of nationwide monitoring activities was screened for filarial nematodes using molecular methods. While *Dirofilaria* sp. and *Setaria tundra* were reported previously,

Cardiofilaria pavlovskyi has now been detected for the first time in the German mosquito fauna. In addition to these characterized filarial species, numerous sequences of non-identifiable filarial origin were discovered. Phylogenetic analyses performed on them were not able to uncover the systematic positions and relationships of their bearers. In some mosquitoes positive for filarial DNA, flavivirus-like RNA sequences could also be detected. The simultaneous detection of filarial nematodes and viruses in mosquitoes is of epidemiological significance since laboratory studies have demonstrated that concurrent ingestion of microfilaria and arboviruses can result in significantly higher viral loads of vector-competent infected mosquitoes and in rendering mosquitoes vector-incompetent for arboviruses to vectors. Further studies on the identity, origin and extent of circulation of the unspecified filarial species and their impact on animal and human health should be made, as well as on the interference of nematodes and viruses in double-infected blood-feeding arthropods.

VEA-O-03

A comparative analysis of subsampling methods to estimate the number of specimens and species in large mosquito samples

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Introduction: Mosquito-borne pathogens are responsible for a huge number of human fatalities every year. Therefore, mosquito surveillance programs gain increasing importance in order to assess the spatial-temporal distribution of vectors and associated agents of diseases.
Objectives: The analysis of samples with thousands of mosquito specimens is a time-consuming and costly process, e.g. in order to enable short-termed decisions for species-specific control measures with insecticides. However, there are no comprehensive subsampling studies available at the moment. Therefore, the objective of this study was the evaluation of five different techniques to accurately subsample large mosquito samples.
Materials & Methods: In total, 23 samples comprising between 400 and 5000 mosquito specimens were analyzed, using 5 different estimation methods: subsampling based on the area, volume and mass of the sample, automatized counting of specimens with the computer software "ImageJ" and random selection of 200 specimens for species identification.
Results: Area-based subsampling of 20% of the total sample resulted in an error of 10% for the number of detected specimens and non-detection of 20% species. Analysing 20% of the volume- or mass based sample leads to 15% error for the number of specimens and 30% for the number of species. The computer software "ImageJ" was a successful approach to estimate the total number of specimens, whereas species detection based on 200 random selected specimens inadequately reflected the real number of species in the sample.
Conclusion: Under the assumption, that an error rate of 10% is an acceptable threshold, subsampling 20% of the area was the most appropriate method to estimate the number of specimens and species in large mosquito samples. Nevertheless, a uniform dispersion of the sample over the gridded paper is challenging and a clustered pattern cannot be completely avoided.

VEA-O-04

Can onchocerciasis be eradicated? Epidemiological studies in three bio-geographic regions of Cameroon and experimental approach in the bovine *Onchocerca ochengi* model

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Introduction: Mass-treatments by ivermectin successfully eliminated onchocerciasis in most of the endemic areas of Latin America and in some isolated foci in Africa. Eradication from the large contiguous areas in Central Africa shall be more challenging.
Objectives: How do the ongoing treatments reduce the transmission in Cameroon? In the bovine *O. ochengi* model we study the acquisition of adult worms, turn-over of microfilariae and density-dependent regulatory mechanisms.
Materials & Methods: *Simulium* vector populations and their *Onchocerca* transmission potentials are monitored in three bioclimatic regions. A herd of Zebu cattle is exposed to natural transmission.
Results: Despite 30 years of annual ivermectin treatments, infective larvae of *O. volvulus* were still found in *S. damnosum* along the Vina du Nord in the Sudan savanna. The Annual Transmission potential is well below 100 infective larvae per man&year and thus close to the threshold. In contrast, transmission has apparently been stopped on the Adamaoua plateau, were the fly-biting density declined and all infective larvae are identified to be *O. ochengi*. Not surprisingly, transmission is still ongoing along the river Menchum in the North-West, where treatments started only in 2012.
In the cohort of Zebu-cattle a cumulative number of ca. 50.000 infective *O. ochengi* larvae is calculated per animal during the 6 years of exposure. Resulting adult worm loads varied from very few (1/3 of animals with 1-5 nodules, i.e. 2 -10 adult worms) to over 1000 nodules in one cow. Microfilarial densities peak at an age of three years and then become largely independent from the load of adult worms. Premunition against L3s and microfilariae is obvious, but strictly species-specific, as shown by high loads of *O. gutturosa* in cattle "putatatively immune" against *O. ochengi*.
Conclusion: In addition to the filaricidal effects of ivermectin, the decline of vector populations reduces the endemicity of *O. volvulus* in man and, to a less extend, of *O. ochengi* in cattle. Environmental changes, zooprophylaxis by increasing cattle stocking density and possibly *Simulium*-toxic pesticides may affect the *Simulium* populations.

Supported by DFG (Re1536/5ff) and BW-Stiftung (BW-plus)

VEA-O-05

The potential for the use of the *Theileria parva* live vaccine cocktail at the wildlife-livestock interface in Uganda: the range of epidemiological situations and antigen gene conservation

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Cattle-to-cattle transmission, by *Rhipicephalus appendiculatus* ticks, plays an important role in the maintenance of *Theileria parva* infections, and the problem is confounded by the presence of a wildlife reservoir for *T. parva*, the African buffalo. Although the use of living parasites is a limiting constraint in production and delivery, the live vaccine - infection and treatment method (ITM), remains the only practical means of providing immunity against *T. parva* in cattle. Two major concerns have constrained deployment of ITM in Uganda: (i) ITM does not always induce complete immunity against heterologous challenge and immunized cattle may be susceptible to challenge with buffalo-derived parasites, (ii) when first introduced into a new geographical location, *T. parva* genotypes from the vaccine that differ from locally circulating populations could be introduced in local tick populations.
We sought to evaluate the likely success of the live vaccination control strategy in Uganda, as determined by serodiagnostic tests and molecular epidemiology of *T. parva* in buffaloes, concomitant data on tick infections and parasite antigen diversity relative to the stocks that comprise the ITM vaccine cocktail.
The results showed a geographically compartmentalised prevalence of *T. parva* in the wildlife reservoir that coincides with the distribution of the tick vector. In co-grazing cattle from regions where *T. parva* is prevalent in buffaloes, we provide evidence of conservation of epitope and antigen gene sequences, which could otherwise contribute to lack of cross immunity between vaccinated animals and local parasites. This is important as buffaloes are known to harbour parasites of higher antigenic diversity than those transmitted between cattle by ticks. The findings suggests that if ITM genetic components are transmitted to local ticks and cattle following immunization, the changes in population genetic structure of *T. parva* may not be major

VEA-O-06
Ecto- and hemoparasites of two small-bodied Malagasy primate species (*Microcebus murinus* and *M. ravelobensis*)

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Parasitic infections of endangered wildlife species are a major concern in conservation biology, due to possible negative effects on the individual but also on the population level. This project focuses on seasonal and species-specific variations in ectoparasite communities and hemoparasites of two small-bodied Malagasy primate species (*Microcebus murinus* and *M. ravelobensis*). Both study species occur sympatrically in the dry deciduous forests in north-western Madagascar and are therefore exposed to the same environmental conditions, but show distinct differences in socioecology. Ectoparasites and blood were collected from trapped individuals over an 11-month period.
Individuals of both mouse lemur species hosted the same species of ticks (*Haemaphysalis* sp.), lice (*Lemurpediculus verruculosus*) and mites (Trombiculidae sp., Laelaptidae sp.) and seasonal and host species-specific differences in sleeping site ecology and sociality were identified as factors influencing ectoparasite patterns. *M. murinus*, sleeping predominantly solitary in tree holes, showed a higher risk of infection with temporary ectoparasites, whereas lice were significantly more often noted in the group-sleeping *M. ravelobensis*. Tick infestation peaked in the late dry season, while lice infections continuously increased over the course of the dry season. Microfilariae were detected in blood smears of both lemurs, with a significantly higher prevalence in *M. murinus* (30.43%, n=69) compared to *M. ravelobensis* (6.59%, n=91) ($p<0.001$).
Parasite frequency in sampled mouse lemur species is shaped by seasonal and socio-ecological factors. The detection of the same ectoparasite species on both mouse lemur hosts requires attention as ectoparasites might serve as vectors for different pathogens, which may pose an additional threat to the infected individual. Ongoing investigations include the screening for protozoan blood parasites (e.g. *Plasmodium* sp.) and genetic identification of detected microfilariae.

VEA-O-07
Molecular interactions of *Trypanosoma cruzi*, triatomines and intestinal bacteria – a review

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Trypanosoma cruzi is the etiologic agent of Chagas disease in Latin America. The flagellates are transmitted via the feces of triatomine bugs. The development of the bugs depends on intestinal obligatory mutualistic symbionts, which are obtained via coprophagy together with other bacteria. Thereby, triatomines harbor a variety of bacteria and fungi in the intestinal tract, but only one species-specific symbiont, an actinomycete. Although blood is sterile, after blood ingestion the expression of genes of antimicrobial compounds – e.g. of defensins, prolixicin, lysozymes, Kazal-type inhibitors and nitric oxide synthases – is upregulated in the cardia and stomach, presumably to exclude a growth of bacteria originating from the skin of the host. The symbiont seems to be resistant to the compounds of the respective triatomine. Not only bacteria but also *T. cruzi* induces the expression of genes of

antimicrobial peptides or compounds. All these immune reactions do not affect *T. cruzi* in a long-term infection. An interaction of *T. cruzi* and bacteria is evident after knocking down antimicrobial peptides that strongly increases the number of bacteria and decreases those of *T. cruzi* However, symbionts should be evaluated separately. During 10 days after infection with *T. cruzi*, the number of the colony forming units of the symbiont is similar in the different regions of the gut of uninfected and infected nymphs. In triatomines captured in the field, an analysis of gut metagenomic DNA also indicates no effects of the infection on the microbiota. Investigating the interactions of *T. cruzi* and intestinal bacteria and symbionts in long-lasting infections of *T. infestans*, different bacteria only develop in infected bugs. Also using high throughput sequencing of region V3-V4 of bacterial 16S rRNA gene, *T. cruzi*-infected triatomines harbour a significantly more diverse bacterial community. Therefore, a *T. cruz* infection induces an intestinal immune response, reducing bacteria, but in long-lasting infections intestinal immunity is suppressed, supporting non-symbiotic bacteria.

Schaub, G.A. et al. (2016) Parasite-vector interactions. In: Walochnik, J; Duchêne, M. (eds.) Molecular parasitology – Protozoan parasites and their molecules. Springer-Verlag, Wien, 431-489, 201

VEA-O-08

Multiantennary N-glycans as unique natural glycodendrimers of the canine heartworm

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Question: The canine heartworm (*Dirofilaria immitis*) is a mosquito-borne parasitic nematode whose range is extending due to climate change facilitating spread of the insect vector. To date little is known about post-translational modifications in this species and whether these are relevant to host immunity.
Methods: In a "four-dimensional" N-glycomic analysis involving two-dimensional HPLC, MALDI-TOF MS and MS/MS in combination with chemical and enzymatic digestions as well as blotting and histochemical experiments, we reveal a nematode glycome of unprecedented complexity.
Results: Not only are N-glycans of up to 7000 Da detected which contain long fucosylated HexNAc-based repeats, but also novel glucuronylated structures. Of the structures which could be resolved completely by our analyses, non-reducing terminal elements such as LacdiNAc, chitobiose, a1,3-fucose and phosphorylcholine were defined on both neutral and glucuronylated glycans which contain up to four antennae. Although some of these features are reminiscent of other filarial nematodes, the occurrence of anionic N-glycans in this phylum was previously never reported.
Conclusion: The N-glycans of *D. immitis* contain a number of features, which may mediate immunomodulation of the host or confer the ability to avoid immune surveillance. Certainly, glycoengineering cell lines to produce recombinant vaccine candidates carrying *D. immitis* glycans,

which in part are conformationally predicted to be like glycodendrimers, will be a challenge as the underlying biosynthetic routes remain to be discovered.

VEA-O-09
Tick abundance in the city of Hanover

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Ticks may act as vectors for a variety of human and animal pathogens. In Germany, the castor bean tick *Ixodes ricinus* is particularly frequent and constitutes the main vector for bacterial and viral zoonotic agents, e.g. *Borrelia* spp., *Rickettsia* spp. and *Anaplasma* spp. The prevalence of these pathogens in Hanoverian ticks has been monitored during the last 10 years. However, not only pathogen prevalence but also tick abundance needs to be taken into account to determine the human and animal infection risk. Therefore, the abundance of ticks in the urban area of Hanover was quantified on a monthly basis from April to October 2017. Questing ticks were collected during the first and second half of each month at 10 different sampling sites in recreation areas in the city of Hanover by the flagging method. At each sampling site, a total area of 200 m² (divided into four 50 m² areas) was sampled. At each visit, one of the four 50 m² areas was sampled on a rotational basis, resulting in 100 m² sampled area per month. In addition, environmental factors like temperature, humidity and vegetation of the sampling area were determined. Tick species differentiation and determination of the developmental stage was carried out based on morphological characteristics. In 2017, more than 1.700 ticks were collected. The monthly tick density at the sites ranged from 0 to 171 ticks/100 m², the average density was 13 ticks/100 m². Tick densities showed a marked peak in May and June at most sites. In addition, a less pronounced peak was recognizable in September. The influence of environmental factors on tick densities is currently being analysed. To obtain reliable data on tick density in the city of Hanover and to gain a more profound understanding of influencing factors, the study will be continued in 2018.

VEA-O-10
Vector role and control of the tick *Dermacentor reticulatus* in Poland

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Questions: Dynamic expansion of *Dermacentor reticulatus* ticks in many areas in central Europe, including western and central Poland, generated a lot of problems, including the spread of canine babesiosis and *Rickettsia* pathogens. Since the beginning of the XXI century, this tick species has became common in central Poland. During last five years we tried to answer key questions regarding the reasons and consequences of tick expansion. We found that *D. reticulatus* constituted the dominant tick species (80%) on livestock and dogs especially in spring and autumn and confirmed its role as a vector of *Babesia canis* and *Rickettsia raoulti*.
Methods: We studied the influence of agricultural practices on the densities of tick in open areas (on pastures, managed meadows and fallow lands). Additionally, in two long-term field experiments, we

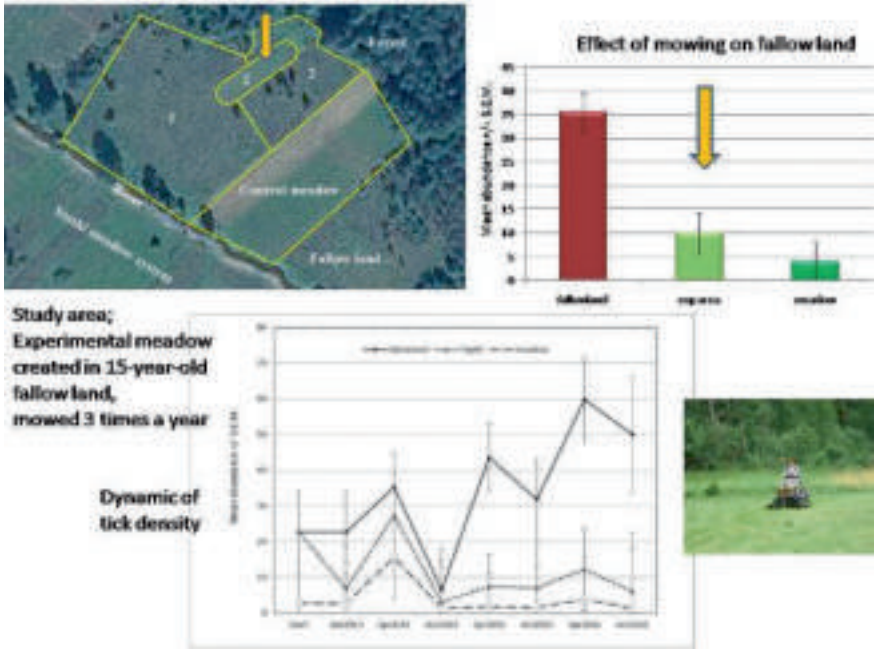
determined the role of regular mowing and cessation of mowing on the densities of ticks. Finally, we determined the impact of spring burning of grasses on tick abundance.

Results: Tick densities were the highest in fallow lands and the lowest on permanently grazed pastures. Cessation of mowing caused continuous increase in tick abundance on newly created fallow lands. On the other hand, regular mowing and burning of grasses in spring resulted with significant decrease in tick densities.

Conclusions: Thus, cessation of agricultural practices may have facilitated the spread of the tick but regular mowing may be implemented as effective control measure for tick population.

Acknowledgements: The study was supported by National Science Centre (NCN) grant Sonata Bis 2014/14/E/NZ7/00153

Figure 1



VEA-O-11

***Amblyomma birmitum* sp. nov. and *Haemaphysalis cretacea* sp. nov.: two new hard tick species in Burmese amber**

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Introduction: Ticks are haematophagous ectoparasites found on terrestrial and semi-aquatic vertebrates. Molecular data suggests the group originated during the Carboniferous, but their fossil record is sparse and restricted to Late Cretaceous deposits or younger.
Objectives: Morphological identification of two Burmese amber ticks in the context of biogeography and possible host relationships.
Materials & Methods: Images for focus stacking were taken using a Leica Z16 APO, Keyence VHX-5000 and 6000 Digital Microscope and X-ray micro CT scanning.
Results: The first fossils assignable to the extant hard tick genera *Amblyomma* (as *Amblyomma birmitum* sp. nov.) and *Haemaphysalis* (as *Haemaphysalis (Alloceraea) cretacea* sp. nov.) are described from the Late Cretaceous Burmese amber of Myanmar. Ixodidae is thought to have split into Prostriata and Metastriata shortly after the end-Permian mass extinction. Prostriata (genus *Ixodes*) prefer mammals today, and may have used groups like cynodonts back in the Triassic. Basal lineages of metastriate ticks (e.g. *Amblyomma*) prefer reptiles, but derived metastriate genera (including *Haemaphysalis*) again prefer mammals. *Amblyomma* and *Haemaphysalis* thus belong to the clade Metastriata; which probably also accommodates two extinct genera: *Cornupalpatum* and *Compluriscutula*. All four fossils are thus only a little younger than published molecular divergence time estimates for the Metastriata lineage. *Amblyomma* has a largely Gondwanan distribution today. It"s predicted radiation time postdates the dissolution of the original Gondwana supercontinent raising questions about how its current distribution pattern was achieved. *Haemaphysalis (A.) cretacea* sp. nov. is anatomically closest to the so-called "structurally primitive" members of the genus.
Conclusion: We confirm the presence of two extant hard tick genera in the Late Cretaceous and offer circumstantial evidence of tick evolution in support of molecular clock predictions.

VEA-O-12

Tick-borne pathogens detection in African cattle by Reverse Line Blot microarray

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Piroplasmoses and rickettsioses are diseases reducing the value of ruminant in the world, therefore having a high economic impact on livestock and farmer life style. Known as vector borne diseases, piroplasmoses (*Babesia and Theileria*) and rickettsioses (*Anaplasma, Ehrlichia and Rickettsia*) are transmitted by ticks which are assumed to be the second most important vectors after mosquitoes to

transmit pathogens to humans and animals. Tick species harbor a broad range of pathogens like viruses, bacteria, protozoa and helminths. Each positive status can be from a primary or chronical infection, or of a carrier stage detectable by thick blood smear or molecular methods. Due to the environmental factors and the vector pressure, cattle can be infested by one or more tick-borne pathogens. The commonly used diagnostic techniques such as conventional or real/time polymerase chain reaction (RT-PCR) have shown limitations both in the detection scope and sample capacity. The PCR-based reverse line blot (RLB) and next generation sequencing are therefore appropriate methods addressing the limitations of the previously used techniques. The latter is a powerful tool in terms of detection rate, number of screened species and samples, including quantification. However this high throughput requires advance laboratory infrastructure and bioinformatics capacity, not achievable in developing countries with poor resources. The "low density" microarray RLB technique is accordingly an approach of choice for laboratories with limited infrastructure to allow rapid, sensitive and specific pathogens detection.
The LCD microarray based hybridization technique has been used for tick-borne pathogen detection on a reusable membrane which requires a long work flow including the treatment of the membrane by the user. In the present study I develop and test a prototype DNA microarray chip for piroplasmids and rickettsiales bacteria to genotyping cattle and tick field samples from Cameroon, Central Africa. After successful testing this chip shall be made commercially available in order to establish them in laboratories of Central Africa. Moreover, our sample size of 1300 cattle and some 3000 ticks from the country"s most populated cattle regions is a valuable asset to estimate the pathogen prevalence per site and enlighten the emergence of new endemic species.

Veterinary parasitology

VPA-O-01

The impact of patent *Fasciola hepatica* infections on individual milk production and fertility parameters in dairy cows

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Infections with *Fasciola hepatica* are associated with high economic losses in the dairy industry. Reductions in milk yield and milk quality as well as impaired fertility were reported for infected herds; however, studies based on individual cow level are lacking. Moreover, infections with *F. hepatica* lead to a suppressed immune response, possibly resulting in a higher susceptibility to other pathogens or udder infections. The objectives of this study were to estimate the association between patent *F. hepatica* infections and i) milk production parameters (milk yield, protein content, fat content), ii) somatic cell counts as an expression of udder health, iii) fertility parameters (calving to first service [CTFS], calving interval [CI], success in first insemination [SFI], 56-day nonreturn rate [NRR56]) in individual dairy cows.
The sedimentation technique was used to determine faecal egg count (FEC) for flukes in 10 gram faeces of 1166 pastured but untreated dairy cows on 17 farms. Linear and generalized linear mixed models were applied to estimate the association between *F. hepatica* infection status (FEC ≥ 1 = infected; FEC = 0 = non-infected) and milk production and fertility parameters, including further

influential factors. No association was identified between *F. hepatica* infection status and milk production parameters or somatic cell counts ($P < 0.05$), albeit infected cows yielded a 0.06 to 0.10% lower protein and fat content compared to non-infected cows.
A significantly higher average CTFS of 4.7 days was detected in *F. hepatica* infected cows ($P = 0.0251$), while other fertility parameters were unaffected. The CTFS interval coincides with the beginning of lactation, a period characterized by the highest susceptibility to stress and disease in dairy cows. Hence, *F. hepatica* infections might have a stronger influence during this period as it was the case for the other fertility parameters.

VPA-O-02
Long-term monitoring of changes in endoparasitic infections as a result of pasture re-wetting in livestock in Northern Germany

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The Northern German federal state Schleswig-Holstein is marked by large, wet grassland and provides pastures for an extensive agricultural usage. Since 2005, a nature conservation program on the peninsula Eiderstedt focusses on re-wetting of these agricultural grasslands and monitors simultaneously the effect on endoparasite infections in sheep and cattle compared to drained pastures. Thus, the aim of the study was to determine whether increased parasite infections occur in grazing livestock on extensive, re-wetted grasslands.

During the monitoring years 2015-2017, a total of 646 fecal samples of cattle and 474 of sheep were analyzed by coproscopical techniques. In each year, samples were collected in April, July and November. Pastures were assigned to three stages of re-wetting: conventionally drained; not drained and extensive farming; not drained and 10.0% of pasture covered by water.
As in the study years 2015 and 2016, data obtained in 2017 determined strongyles as the most frequent endoparasites [cattle: 39.5% (86/218); sheep: 61.1% (102/167)] followed by *Eimeria* spp. [cattle: 11.1% (24/218); sheep: 30.5% (51/167)]. Regarding lungworms, larvae were solely found in fecal samples of sheep [7.8% (13/167)]. Eggs of rumen flukes were present in 25.7% (56/218) of fecal cattle samples, but solely in 1.2% (2/167) of sheep. *Fasciola hepatica* eggs were found in 6.4% (14/218) of cattle samples and 4.2% (7/167) of sheep. By comparison with results obtained in 2016, *F. hepatica* prevalences in cattle remained consistent between 2016 and 2017 (6.0% vs. 6.4%), contrary to higher prevalences in 2015 (17.6%). In sheep, significantly decreasing liver fluke prevalences were determined between 2015, 2016 and 2017 (21.6% vs. 13.7% vs. 4.2%).
Further analyses on the effect of seasonality as well as the long-term effects of re-wetting on endoparasite infections are in progress and results will be presented.

VPA-O-03

***Coccidiosis* and other parasitosis in milk livestocks from Lower Saxony: effects of different husbandry systems**

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Introduction: The System Analysis Milk project aims to analyse effects of stable and pasture husbandry on economy and animal welfare. The parasitological analyses focusd on eimeriosis, cryptosporidiosis, gastrointestinal nematodes (GIN) and presence of milk antibodies against *Fasciola*, *Dictyocaulus* and *Cooperia*.
Objectives: The study aimedd at (i) characterisation of parasite populations in both systems, (ii) identification of risk factors for particular parasitoses and (iii) improved recommendations to prevent losses due to parasitoses.
Materials & methods: In spring (3 weeks after turn-out) and autumn (around housing) 2015/16, faecal samples from in total ~2800 calves and heifers from 46 farms were examined using *Cryptosporidium* coproantigen tests and Mini-FLOTAC. After flotation, *Eimeria* species were identified. In addition, more than 2700 milk tests were collected and analysed for presence of antibodies using a multi-plex Luminex system.
Results: A significantly higher number of oocysts/g faeces was observed in grazing heifers in autumn than in spring and in the stable at any time point. The most common *Eimeria* species were *E. zuernii* and *E. bovis*. Faecal egg counts for GIN in heifers were low on pasture (median 5, 90% percentile 60) but still significantly lower for non-grazing animals (median 0, 90% percentile 0). There were no significant differences between the seasons. Nearly all calves were kept without access to pasture. The overall *Cryptosporidium* prevalence was 22.9% without significant differences over time but *Cryptosporidium* positive calves were significantly younger than negative calves.
Conclusions: Although access to pasture increased prevalence of parasitoses, the effects were unexpectedly small. The farmers dewormed the heifers on pasture very regularly without preceding diagnosis. The low GIN burden shows that farmers should be advised to perform diagnosis and targeted selective anthelmintic treatment to avoid resistance selection.

VPA-O-04

Genetic and transcriptomic characterization of the P-glycoprotein gene family in *Parascaris* sp.

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Introduction: Infections with the horse roundworm *Parascaris* sp. pose a major threat to foal health and anthelmintic treatment remains the most common control strategy. The development macrocyclic lactone (ML) resistance has become a worldwide obstacle to maintaining equine health.
Deciphering the mechanisms of ML resistance in nematodes is challenging, presumably due to a multigenic nature of the trait. P-glycoproteins (Pgps) were proposed as a major contributor in several ML resistant parasites. The nematode Pgp family has undergone expansion through duplication and to date it is unclear which of the various Pgps are involved in ML resistance. So far, the identification and characterization of responsible Pgps has focussed on randomly chosen candidates, most likely leading to a bias.
Objective: This study aimed to characterize a complete ascarid Pgp gene family exemplarily using *Parascaris univalens*.
Materials and Methods: Two independent transcriptomes were screened for putative Pgps using tBLASTn. cDNA sequences were then improved using RT-PCR and Sanger sequencing. Deduced protein sequences were compared to the Pgp repertoire of other nematodes by phylogenetic analysis.
Results: Only one Pgp contig with a complete open reading frame (ORF) was identified in the transcriptomes but 10 full-length Pgp ORFs were obtained through transcriptome-guided RT-PCR. Comparison of actual cDNA sequences and their corresponding contigs revealed errors due to gaps, INDELs and incomplete assembly of non-overlapping contigs. Phylogenetic analysis revealed a Pgp lineage currently exclusive to ascarids, *Asu*MRP3a and *Pun*iPgp-17, as well as a Pgp without an orthologue.
Conclusion: The Pgp repertoire in *P. univalens* largely resembles that of other parasitic nematodes. However, the diversification of Pgps in nematodes remains an active process in recent evolutionary history, which carries possible implications for the development of multi-drug resistance in light of the strong selection pressure asserted by current worm control strategies.

VPA-O-05
Pathological findings in intestines of grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) from the North and Baltic Seas associated with acanthocephalan infections

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Grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) are the most common seal species in the North and Baltic Seas and final hosts of various parasites. Acanthocephalan infections are increasingly observed since sampling started in 1998. Both seals´ small intestines are infected with *Corynosoma strumosum*/*magdaleni*. Baltic grey seals regularly display severe colonic *Corynosoma semerme* infections associated with ulcers and thickened intestinal walls as part of the Baltic Seal Disease Complex (BSDC), formerly including reproductive tract and endocrinal lesions. Pathogenesis and correlation of infections with these alterations are unknown yet.

This study focuses on lesion differences and parasitic distribution per seal species. Samples of infected grey seal (n=67) and harbour seal (n=257) intestines collected in Estonia and by the German and Polish monitoring systems from 1998-2016, were taken for histopathology. Harbour seals mainly originated from the North Sea (n=246), grey seals from the Baltic Sea (n=55). The harbour seal sex ratio was almost equal (131 females/126 males), while male grey seals exceled the females (46 males/21 females).
Both species´ small intestines were mildly-moderately infected. Grey seals showed more moderate and severe colonic infections (n=18; n=13) than harbour seals (n=7; n=4). Young harbour seals displayed a mural, granulomatous, eosinophilic/lymphoplasmacellular enteritis. Adult and young Baltic grey seals showed a chronic, erosive/ulcerative, eosinophilic/lymphoplasmacellular colitis and tunica muscularis hypertrophy.
Corynosoma spp. infections were not fatal, but may affect seal health negatively. They presumably induce the primary lesion, promoted by the Baltic seals´ pollutant-impaired immune system. The grey seal lesions´ severity stands out notably compared to harbour seals. Findings indicate still prevailing symptoms of the BSDC, that acanthocephalans may be suitable health indicators and the colon a target organ.

VPA-O-06
Reaction of immune cells within the intestine of thinlip mullet, *Liza ramada* (Pisces), in response to micro- and macro-parasitic infection

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The Mugilidae, commonly known as grey mullets, are among the most ubiquitous teleost families inhabiting coastal waters. There are only a limited number of studies that have examined the concurrent infection of fish by myxozoans (microparasites) and by helminths (macroparasites). The current study, therefore, set out to investigate the immune mechanisms in play within the intestines of *Liza ramada,* co-infected with micro- and macro-parasites. The intestines of 58 thinlip mullet collected from Comacchio"s lagoons in northern Italy were investigated by various histopathology- and electron microscopy-based techniques. Pieces of both infected and uninfected intestinal tissue were excised and then prepared following standard methods for immunohistochemical and ultrastructural evaluation. Eighty-five percent of the fish harboured at least one of the following intestinal parasites: *Myxobolus mugchelo* (Myxozoa), *Neoechinorhynchus agilis* (Acanthocephala), *Haplosplanchnus pachysomus* and *Dicrogaster contractus* (Digenea). Parasitic co-infection was common with 36% of fish co-infected with all of the parasites listed above, 28% with myxozoans and digeneans, 15% with myxozoans and acanthocephalans, and 2.5% with all three helminth species. At the site of attachment of helminths, several mast cells (MCs), rodlet cells, mucous cells, a few neutrophils and macrophages were observed within the epithelium. Conspicuous plasmodia of *M. mugchelo* were seen encysted, principally, within the muscle and submucosa layers of the intestine, while numerous free spores were found scattered among the epithelial and connective tissues of the mucosa. Degranulating MCs in close proximity to the myxozoans were frequently observed. From this study, MCs were the most dominant immune cell encountered within the intestines of infected *L. ramada*, where they appear to play an active regulatory role in coordinating the functions of the host's innate immunity.

VPA-O-07
Involvement of rodlet cells in fish organs infected with metazoan parasites

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Rodlet cells (RCs) are characterised by a distinctive cell cortex and conspicuous rodlet-shaped inclusions, which account for their name. They are enigmatic cells found within the viscera and epithelia of virtually every marine and freshwater inhabiting teleost fish. We present data regarding the response of RCs to several parasite taxa with comments on their presence at the host-parasite interface and their ultrastructure. Studies include information stemming from histochemical, immunohistochemical and ultrastructural-based investigations in infected and uninfected tissue collected from several orders of fish. In European eels, *Anguilla anguilla*, infected with the digeneans *Deropristis inflata* and *Helicometra fasciata* and the acanthocephalan *Acanthocephalus clavula,* RCs within the gut epithelium were found in higher numbers at the sites of parasite attachment. Within these RCs, the inflammatory peptides lysozyme and inducible-nitric oxide synthase were found. In some sections of the eel"s intestine, a few intact RCs were observed free in the lumen to which, interestingly, unclassified bacteria were commonly observed in intimate contact with the plasma membrane. In other case studies, RCs were also seen at: the site of parasite attachment in the intestines of tench, *Tinca tinca,* infected with the cestode *Monobothrium wageneri*; in the liver and pancreas of minnows, *Phoxinus phoxinus,* infected with the nematode *Raphidascaris acus*; and, in brain of minnows infected with the metacercariae of the digenean *Diplostomum phoxini*. Numerous RCs were also noticed within the gill epithelia of bream, *Abramis brama,* parasitised by the crustacean copepod, *Ergasilus sieboldi*, and in those of European seabass, *Dicentrarchus labrax,* infected with the monogenean *Diplectanum aequans.* RCs function as immune cells responding to sites of metazoan parasite infection, evidenced by their localised increased number.

Workshop • Tungiasis

WOT-O-O1
***Tunga penetrans* infections among animals and humans in East Africa**

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Penetration of female *Tunga penetrans* into the skin of mammalian hosts causes a debilitating but extremely neglected tropical disease called tungiasis. Zoonotic tungiasis which is endemic in Latin American and sub Saharan countries causes a significant morbidity in humans and animals particularly among poor communities. In East Africa, reliable geographic information regarding prevalence, intensity and severity of tungiasis is barely available. To date, there are few studies on the occurrence

of tungiasis in Uganda, Kenya, Tanzania, Ethiopia and Madagascar. Cross sectional studies suggest high prevalence of human infections which approach 100% among some study communities where a wide range of risk factors associated with poverty and unhygienic living conditions prevail. Highest infection intensities and prevalence are reported among children, disabled and the elderly persons. Animals are central to the transmission dynamics of tungiasis but studies on their role in the epidemiology of tungiasis are very scarce. A study in Uganda identified pigs, dogs, cats and goats as animal reservoirs for *T. penetrans* and reported increased odds of occurrence of human infections in households and villages with infected animals. Heavy sand flea infections and secondary bacterial infections often cause severe morbidities in both humans and animals, which cause variable degrees of body deformities and reduced productivity, sometimes even leading to death. Unfortunately, effective treatment and prophylactic options for human and animal tungiasis are limited and inaccessible to the affected communities while sustainable control strategies are generally lacking. Due to high prevalence and severe disease among humans and animals, tungiasis contributes to the vicious cycle of poverty among endemic communities. As a zoonosis, tungiasis calls for a multidisciplinary approach for effective control including systematic implementation of recently described effective treatment options for humans and animals.

WOT-O-O2
Control of tungiasis: experiences and challenges

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Introduction: Tungiasis is a tropical parasitic skin disease caused by the penetration of the female sand flea *Tunga penetrans* into the epidermis of its host, usually on the feet. Although widespread in resource-poor communities in sub-Saharan Africa, South America and the Caribbean and associated with important morbidity control has never been attempted in a systematic manner. Since effective and safe treatment is not available in the endemic areas, patients take rescue to the application of toxic compounds and/or try to remove embedded sand fleas with inappropriate non-sterile instruments, a health hazard by itself.
Objectives: To give an overview on methods through which morbidity control of tungiasis may be achieved.
Methods: All studies/projects aiming at morbidity control of tungiasis realized in the last ten years were reviewed.
Results: Studies in Brazil and Madagascar have proved that the daily application of a repellent based on coconut oil is an effective prevention measure. Recent studies in Kenya and Uganda have shown that the topical application of a two-component dimeticone is a highly effective treatment since sand fleas that were not killed after a single application of the dimeticone were interrupted in their natural development and stopped to expel eggs. Systematic treatment with dimeticone could result in the interruption of indoor transmission. In communities in costal Kenya tungiasis was controlled by treating infected individuals with a mixture of neem and coconut oil together with spraying floors with a watery neem solution.
Conclusion: Different approaches allow the control of tungiasis-associated morbidity in resource-poor communities. However, elimination of tungiasis requires a One-Health Approach.

WOT-O-O3
Prevalence, intensity and risk factors of tungiasis in Kilifi County, Kenya: I. Results from a community-based study

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Question: Tungiasis is a neglected tropical disease caused by female sand fleas (*Tunga penetrans*) embedded in the skin. The disease is endemic along the Coast of Kenya with a prevalence ranging from 11% to 50% in school-age children. In this study we identified important risk factors for the occurrence of tungiasis and severe disease.
Methods: In a cross-sectional study 1,086 individuals from 233 households in eight villages in Kilifi County were investigated. Study participants were examined systematically and the presence and severity of tungiasis were determined using standard methods. Demographic, socio-economic, environmental and behavioral risk factors of tungiasis were assessed using a structured questionnaire. Data were analyzed using bivariate and multivariate regression analysis.
Results: The overall prevalence of tungiasis was 25.0% (95% CI 22.4–27.5%). Age-specific prevalence followed an S-shaped curve, peaking in the under-15 year old group. In 42.5% of the households at least one individual had tungiasis. 15.1% of patients were severely infected (≥ 30 lesions). In the bivariate analysis no specific animal species was identified as a risk factor for tungiasis. Multivariate analysis showed that the occurrence of tungiasis was related to living in a house with poor construction characteristics, such as mud walls (OR 3.35; 95% CI 1.71-6.58), the number of people per sleeping room (OR = 1.77; 95% CI 1.07–2.93) and washing the body without soap (OR = 7.36; 95% CI 3.08–17.62). The odds of having severe tungiasis were high in males (OR 2.29; 95% CI 1.18-44.6) and were very high when only mud puddles were available as a water source (OR = 25.48; 95% CI 3.50–185.67) and lack of water permitted washing only once a day (OR = 2.23; 95% CI 1.11–4.51).
Conclusions: The results of this study show that in rural Kenya characteristics of poverty determine the occurrence and the severity of tungiasis. Intra-domiciliary transmission seems to occur regularly.

WOT-O-O4
Very severe tungiasis : a life-threatening condition

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Introduction: Tungiasis is a neglected tropical disease caused by female sand fleas (*T. penetrans*) embedded in the skin. By definition, tungiasis is a self-limiting infection. However, in endemic settings constant re-infection is the rule and parasite load and severity of disease gradually accumulate over time. Infections with hundreds of embedded sand fleas immobilize the patient, lead to malnutrition, anaemia, cachexia and eventually death.
Objectives: To describe the environmental characteristics, the medical history and the clinical pathology associated with very severe tungiasis.

Methods: During a study on parasitic skin diseases in Vaupés Department, Amazon lowlands, Columbia, five patients with very severe tungiasis were identified and examined.
Results: Patients had between 250 and 1300 embedded sand fleas. The feet were predominantly affected, but clusters of embedded sand fleas also occurred at the ankles, the knees, the elbows, the hands, the fingers and around the anus. The patients were partially or totally immobilized. Four patients were cachectic, one patient presented severe malnutrition. One patient needed a blood transfusion due to severe anaemia. All patients showed a characteristic pattern of pre-existing medical conditions which exposed the patients to constant re-infection. All patients were extremely poor and lived secluded from the community. In all cases intradomiciliar transmission was very likely.
Conclusions: Although not mentioned in the literature, very severe tungiasis still occurs in settings, where patients do not have access to health care and are stricken in a net of pre-existing illness, culturally determined risk behavior and poverty.

WOT-O-O5
High infection frequency with *Wolbachia pipientis* and potentially transmissible *Rickettsia bellii*-like bacteria in *Tunga penetrans* from Uganda and Kenya

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Introduction: Tungiasis caused by skin penetrating female sand fleas is a severely neglected tropical zoonosis. *Tunga penetrans* is endemic in Latin America and Africa but data on genetic diversity of fleas and associated endosymbionts is limited. In some but not all *T. penetrans* populations, *Wolbachia* were detected and there is one report on presence of *Rickettsia felis*.
Objectives: The study aimed to explore the molecular diversity of *T. penetrans* and its endosymbionts in East Africa.
Methods: *T. penetrans* collected from different hosts and regions in East Africa were genotyped by sequencing partial *cox2* and *cytb* genes. Presence of Rickettsiales was determined using *Wolbachia* and *Rickettsia* specific *gltA* PCRs. In maximum likelihood phylogenetic analyses, 16S rRNA and some *groEL* and *coxA* sequences were used.
Results: Identical *cytb* and *cox2* sequences were obtained for 80 fleas. The cox2 was identical to a sequence from Madagascar and showed only few differences to sequences from South America. PCRs targeting *gltA* and 16S rRNA genes identified two unrelated *Wolbachia* genotypes occurring with frequencies of 100% (wTpen1; 95% confidence interval [CI] 92.2-100%) and 90.3% (wTpen2; 95% CI 75.1-96.7%). While the previously reported wTpen1 genotype was located in a multi-locus phylogenetic analysis at a relatively basal position of the tree, the newly described wTpen2 genotype clustered with the supergroups C, D, and F including many obligate mutualistic endosymbionts. *Rickettsia belli*-like bacteria very closely related to mildly pathogenic tick-born *R. belli* were detected in 76.3% (95% CI 65.8-84.2%) of the fleas.

Conclusions: It remains unclear if these maternally transmitted bacteria improve host fitness or cause phenomena such as male killing, feminisation or cytoplasmic incompatibility. Release of bacterial lipopolysaccharide from degenerating fleas will presumably aggravate inflammation and *R. belli* transmission by *T. penetrans* cannot be excluded.

GRK 2046 Workshop • Parasite-microbiota interaction

WSG-O-01

Of worms, germs and men – from nature to the clinic

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The gastrointestinal (GI) tract of vertebrates is inhabited by a vast array of organisms, that is, the microbiota and macrobiota. The former is composed largely of commensal microorganisms, which play a vital role in host nutrition and maintenance of energy balance, in addition to supporting the development and function of the vertebrate immune system. By contrast, the macrobiota includes parasitic helminths, which are mostly considered detrimental to host health via a range of pathogenic effects that depend on parasite size, location in the GI tract, burden of infection, metabolic activity, and interactions with the host immune system. Sharing the same environment within the vertebrate host, it is plausible that the GI microbiota and parasitic helminths interact with each other, and that the results of such interactions may impact, directly or indirectly, on host health and homeostasis. For instance, parasitic helminths and microbiota compete for host nutrients while, in parallel, the known immune-modulatory properties of a range of parasites may translate into modifications of mucosal and systemic immunity to the resident bacteria. The complex relationships occurring between parasitic helminths and microbiota have long been neglected; however, recent studies pointing towards a role for these interactions in the overall pathophysiology of helminth disease, as well as in parasite-mediated suppression of inflammation. Nevertheless, current knowledge of helminth-microbiota interplay relies heavily on studies conducted in rodent models of infection and disease. This presentation will provide an overview of our recent efforts to characterise the qualitative and quantitative changes in gut microbial profiling, as well as in relative abundance of individual microbial species, in humans infected by parasitic helminths (nematodes), under both natural and experimental settings.

WSG-O-02

Secreted products of the intestinal roundworm *Ascaris suum* impact bacterial growth and biofilm formation

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Introduction: Soil-transmitted helminth infections are widespread amongst humans and animals. Ascariasis is a considerable public health burden as the most prevalent intestinal nematode infection of man and a leading infection in pigs. Inhabiting the small intestine of its host, *Ascaris suum* lives in contact with numerous microbial species which may pose infectious challenges for the worms, yet the nature of the interactions between intestinal nematodes and bacteria are poorly understood.
Objectives: We aimed to investigate the antimicrobial components and activities of excretory-secretory products (ESP) and body fluid (BF) of *A. suum*.
Materials & Methods: Different life stages of *A. suum* were kept in culture, including *in vitro*-hatched L3, lung-stage L3, L4, and adult stages and their ESP collected and filtered. Additionally, BF from adult males was collected from the nematode pseudocoelem, filtered, and analyzed. Nematode products were characterized by liquid chromatography-tandem mass spectrometry. Growth inhibition of diverse bacterial strains by nematode products was assessed using the radial diffusion assay, while anti-biofilm activity against *Escherichia coli* was assessed using the crystal violet and macrocolony biofilm assays. Effects of nematode products on *in vivo*-relevant bacterial strains were also assessed.
Results: Several proteins and peptides with known and predicted innate immune activities were detected in nematode products, including antimicrobial peptides, glycosyl hydrolase enzymes, and c-type lectin domain-containing proteins. Correspondingly, nematode products displayed diverse antimicrobial activities and impacted the growth of in vivo-relevant bacterial strains.
Conclusion: These results indicate that *A. suum* uses several factors for broad-spectrum defense in order to shape its microbe-rich environment.

WSG-O-03
Gut microbial changes in mice experimentally infected with *Schistosoma mansoni*

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Approximately 230 million people worldwide are infected with *Schistosoma* spp. and the resulting tropical disease, schistosomiasis, is second only to malaria in terms of its socioeconomic and public health impact. One under-explored aspect of schistosomiasis is its impact on the microbiome. Here, we assessed the qualitative and quantitative differences between the gastrointestinal (GI) microbial profiles of cohorts of 10 laboratory mice experimentally infected with *Schistosoma mansoni* and 10 uninfected control mice, at 28 and 50 days post-infection (p.i.). Microbial DNA was extracted from luminal contents of the small (SI) and large intestine (LI), followed by high-throughput sequencing of the bacterial 16S rRNA gene and bioinformatics and biostatistical analyses. Significant differences in SI and LI gut microbial composition were observed between infected and uninfected mice, and between sampling time-points. In particular, at D50 p.i., a significant reduction in alpha diversity was observed in infected mice, when compared with mice at D28 as well as to non-infected mice. On the contrary, microbial beta diversity increased in infected mice at D50 p.i. compared with D28 p.i. (SI, $P = 0.048$; LI, $P = 0.015$). Amongst the microbial taxa that were significantly affected by *S. mansoni* infection, the Verrucomicrobia, Lactobacillaceae and Bacteroidaceae were increased in infected mice compared with uninfected controls whereas the opposite trend was observed for Turicibacterales and Clostridiaceae. Functional predictions associated with the microbial profiles of schistosome-infected and uninfected

mice revealed that the majority of bacterial taxa affected by the presence of parasites were linked to immune-regulatory activities. A deeper understanding of the whole complement of interactions occurring among the parasite, the gut microbiota and the host immune system may assist in elucidating the pathophysiology of schistosomiasis, and would provide a basis for the discovery and development of novel intervention strategies based on the manipulation of the gut microflora.

Figure 1

WSG-O-04

Metabolism of high-density *Giardia* foci of infection in the mouse small intestine: interactions with the host microbiome

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Our current understanding of *Giardia* metabolism in the host is largely inferred from co-culture with mammalian cell lines. Here we present this new evaluation of Giardia, host, and commensal bacterial metabolism associated with in vivo foci. We recently developed bioluminescent imaging (BLI) methods to visualize parasite metabolism directly in live animal hosts. Next, we used bioluminescent imaging (BLI) to precisely select gastrointestinal samples with discrete Giardia foci to interrogate in vivo parasite, host, and microbial metabolism using metagenomic, transcriptomic, and metabolomic strategies. We discovered that parasites colonize and encyst in dense foci in the small intestine early during infection. These high-density regions of parasite colonization may result in localized pathology to the epithelium. Encystation occurs during infection as trophozoites reach a local threshold density and may be subject to host inflammatory responses. Metabolism within the foci is defined by upregulation of encystation and oxidative stress responses. Specifically, relative to Giardia in culture, gene expression in foci was enriched for glycolytic, oxidative stress response, and encystation-specific lipid and cyst wall biosynthesis pathways. With respect to host microbiota and localized oxidative stress, we found a systemic dysbiosis of bacterial commensal diversity throughout the gut during Giardia colonization, with a marked increase in aerobic Proteobacteria and a decrease in Clostridiales. Lastly, metabolomic analyses indicate that glycerophosphoinositols and other lipid metabolites were altered in gut regions associated with foci of encysting Giardia. This new window into the temporal

and spatial variations of gene expression during in vivo Giardia infection in the host gastrointestinal tract enables future mechanistic studies of host-specific Giardia physiology.

WSG-O-05

Role of the REL2/Imd signaling pathway and the host-microbes interplay in the vectorial capacity of *Anopheles gambiae*

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Functioning of an organism is allowed due to the collective effort of multiple individual cells organized into tissues, organs and systems. Furthermore, their function is fine-tuned through activity of various molecules and intracellular signaling pathways. Although increasing evidence suggests the contribution of different tissues to immunity, the information remains limited. In the mosquito, hemocytes (blood cells), fat body and midgut were previously suggested to encode genes involved in infection by malaria parasites and bacteria. Additionally, experimental activation of intracellular signaling pathway REL2/Imd was found to be curbing the development of human malaria parasites, *Plasmodium falciparum*. However mechanisms behind malaria killing in the mosquito remain largely unknown. With an aim of identifying mechanisms that determine the REL2 specificity of *Plasmodium* killing in the mosquito, we established loss-of-function mutants of the NF-κB transcription factor REL2 using CRISPR-Cas9 system (REL2-/-). Using these mutants, we began addressing the involvement of different tissues in the immune response of *Anopheles gambiae* mosquitoes and the role of REL2 pathway in these responses. Our approach relies on the use of transcriptomics and RNA in situ hybridization to uncover transcriptional profiles of hemocytes, fat body and midgut. Preliminary analyses identified first set of REL2 effector genes and indicated on both tissue-specific and ubiquitous gene expression. Surprisingly, we also observed that the susceptibility of REL2-/- mutants upon *P. falciparum* infection depends greatly on the gut microbiota. Therefore, we are set to identify if changes of microbiota can affect *Plasmodium* development and mosquito susceptibility to the parasite. We believe that this study will allow us to better understand REL2 signaling, will advance our knowledge about vector-pathogen interactions and may identify new targets to combat malaria.

WSG-O-06

Metabarcoding the bacterial and eukaryotic (micro-)biome reveals an abundance of unknown intestinal inhabitants

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Not all organisms that inhabit a host are necessarily parasitic. This trivial insight is intuitively accepted for bacteria but not for eukaryotes. I here report on the diversity of eukaryotes inhabiting or passaging the intestines of a select group of mammals.
To comprehensively assess eukaryotic content in the intestine or droppings of zebras, hyenas and wolves, we used a customized set of 48 primer pairs in PCR reactions. We sequenced the resulting amplification products representing dietary items, passaging material, and the bacterial and eukaryotic microbiome.
We found that sensitivity of detection and specificity of taxonomic assignments was improved by the combination of multiple amplicons compared to any individual marker gene. Diversity of eukaryotes in the intestines of wildlife exceeded that reported in humans. While fungi (Ascomycota and Basidiomycota) dominated the eukaryotic microbiome, a previously unobserved diversity was also found for basal Apicomplexa and Ciliophora for example. Interestingly, known parasites (i.e. helminths) recorded simultaneously using our metabarcoding and classical coprological methods could validate quantitative assessments based on our metabarcoding results for individual species. On a broader scale, we demonstrated how ancestral state reconstructions and extrapolations in a phylogenetic context can be used to deduce the most likely status of taxa identified by marker sequences belonging to the diet or microbiome respectively. Predictions of the mode of a symbiotic relationship with a host (friend or foe) with this approach is bound to fail due to the scarcity of reports on mutualistic intestinal eukaryotes in the literature and databases.
We conclude that insufficient knowledge of the eukaryotic component of the mammalian intestinal ecosystem currently exists to classify the majority of eukaryotes as parasites. Parasitologists working on intestinal eukaryotes should consider concepts of symbiosis: lifestyles ranging from parasitic via commensal to mutualistic.

Poster
Biochemistry

BIO-P-01

Identification of anti-microbials and their mode of action against persisting stages of *Toxoplasma gondii*

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Toxoplasma gondii is estimated to infect a third of the human population world-wide and has a 50% prevalence in Germany. These chronic infections are caused by the encysted bradyzoite stage of the parasite. While existing chemotherapeutics are effective against the acutely virulent tachyzoite stage of *T. gondii* they do not clear chronic infections and are accompanied by serious side effects. This shortcoming along with the lack of vaccines against *T. gondii* in humans demands development of new chemotherapeutic intervention strategies. With this purpose many essential proteins were identified and considered drug targets in *T. gondii*. However, development of corresponding antimicrobials has proven difficult. As a solution to this problem anti-microbial compound libraries were made available to the scientific community. The recently published MMV Pathogen Box contains 29 compounds that have been shown to be effective against *T. gondii* tachyzoites at concentrations below 1 µM. Their activities and modes of action however remain unclear. We aim to identify compounds that target bradyzoites and identify their modes of action using a comprehensive mass spectrometry-based metabolomics platform consisting of in house gas- and liquid chromatography coupled mass spectrometry and reverse genetics.

To that end we established and optimized a plate-based and medium throughput compatible assay using tdTomato-expressing parasites. Our data confirm reported IC50 values of well characterized drugs such as pyrimethamine and ciprofloxacin. We are now extending this assay to enable screens against bradyzoites and screens for delayed-death phenotypes. The resulting data will inform follow-up metabolomics and reverse genetic experiments.

BIO-P-02

Two different thioredoxin reductases in one amoeba: the extraordinary thioredoxin-linked redox system in *Acanthamoeba castellanii*

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Introduction: *Acanthamoeba castellanii* is a free-living protist which feeds on bacteria. However, it can also cause infections in man, mainly *Acanthamoeba* keratitis (AK) and granulomatous amoeba encephalitis (GAE). Infections with *A. castellanii* are difficult to treat, demanding enhanced efforts for a better understanding of its physiology in order to develop new treatment options. A singularity in acanthamoebae is the co-existence of two different thioredoxin reductases (TrxRs): one of the small bacterial type, and one of the large eukaryotic type. As redox systems in parasites provide good

targets for antiparasitic therapy, we speculate that either one or both of *A. castellanii*"s TrxRs could be a valid drug target.
Objectives: The major objective of this project is to characterize both TrxRs of *A. castellanii* and their physiologic roles. They, their substrates, and interaction partners, e.g. thioredoxins and peroxiredoxins, will be expressed in *E. coli* and tested for activity. It will be further attempted to genetically knock-down the expression of both TrxRs in order to assess their physiological importance. After knock-down of TrxRs, acanthamoebae will be tested in an *in vitro* cornea model for pathogenicity.
Materials & Methods: Thioredoxin reductases will be expressed in *E. coli* and characterized biochemically. Further, other proteins involved in the antioxidant defense will be expressed and characterized. Expression of TrxRs will be quantified under various conditions (growth phase, ambient oxygen levels) at the mRNA and/or protein levels by RT-qPCR and western blot. The localization of the TrxRs within acanthamoebae will be determined using immunofluorescence microscopy. Down-regulation of TrxR expression will either be achieved by siRNA or antisense mRNA techniques.
Results: The project is still in an early phase, but both TrxRs were confirmed to be expressed in *A. castellanii*.
Conclusion: None so far.

BIO-P-03
The cytosolic glyoxalases of *Plasmodium falciparum* are dispensable during asexual blood-stage development

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The enzymes glyoxalase 1 and 2 (Glo1 and Glo2) are ubiquitous proteins and can be found in most eukaryotes and several prokaryotes. They catalyze the glutathione-dependent conversion of 2-oxoaldehydes to 2-hydroxycarboxylic acids. The accumulation of harmful 2-oxoaldehydes, such as glucose-derived methylglyoxal, can lead to the formation of advanced glycation endproducts (AGE). The genome of the malaria parasite *Plasmodium falciparum* encodes for four glyoxalases: the cytosolic enzymes *Pf*Glo1 and *Pfc*Glo2, the apicoplast enzyme *Pf*tGlo2 and an inactive Glo1-like protein that also carries an apicoplast-targeting sequence. *P. falciparum*-infected erythrocytes were shown to consume up to 75 times more glucose than uninfected erythrocytes resulting in an increased production of methylglyoxal and D-lactic acid. Inhibition or knockout of the Plasmodium glyoxalases was therefore hypothesized to lead to an accumulation of 2-oxoaldehydes and advanced glycation end-products in the host-parasite unit and to result in parasite death. Here, we generated the clonal 3D7 knockout lines Δ*pf*glo1 and Δ*pfc*glo2 using the CRISPR-Cas9 system. Although 3D7 Δ*pf*glo1 knockout clones showed an increased susceptibility to external glyoxal, all 3D7 Δ*pf*glo1 and Δ*pfc*glo2 knockout lines were viable and lacked a significant growth phenotype under standard growth conditions. Furthermore, the lack of *Pfc*Glo2, but not *Pf*Glo1, increased gametocyte commitment in the knockout lines. In summary, *Pf*Glo1 and *Pfc*Glo2 are dispensable for asexual blood stage development while the

loss of *Pfc*Glo2 may induce the formation of transmissible gametocytes. These combined data show that PfGlo1 and PfcGlo2 are most likely not suited as targets for selective drug development.

BIO-P-04

A single-cysteine mutant and chimeras of essential *Leishmania* Erv can complement the loss of Erv1 but not of Mia40 in yeast

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Mia40/CHCHD4 and Erv1/ALR are the essential key players for oxidative protein folding in the mitochondrial intermembrane space of yeast and mammals. In contrast, many protists including important apicomplexan and kinetoplastid parasites lack Mia40, and the Erv homolog from the model parasite *Leishmania tarentolae* (*Lt*Erv) was shown to be incompatible with *Saccharomyces cerevisiae* Mia40 (*Sc*Mia40). Here we addressed structure-function relationships of *Sc*Erv1 and *Lt*Erv and their compatibility with the oxidative protein folding system in yeast using chimeric, truncated and mutant Erv constructs. Chimeras between the N-terminal arm of *Sc*Erv1 and a variety of truncated *Lt*Erv constructs were able to rescue yeast cells lacking *Sc*Erv1. Yeast cells were also viable when only a single cysteine residue was replaced in *Lt*ErvC17S. Thus, the presence or position of the C-terminal arm and the kinetoplastida-specific second (KISS) domain of *Lt*Erv do not interfere with its function in the yeast system, whereas a relatively conserved cysteine residue before the flavodomain renders *Lt*Erv incompatible. The question whether parasite Erv homologs might also exert the function of Mia40 was addressed in another set of complementation assays, but neither the KISS domain nor other truncated or mutant *Lt*Erv constructs could rescue yeast cells lacking *Sc*Mia40. The general relevance of Erv and its candidate substrate small Tim1 was analyzed for the important related parasite *L. infantum*. Repeated unsuccessful knockout attempts suggest that both genes are essential in this human pathogen and underline the potential of mitochondrial protein import pathways for future intervention strategies.

Cell biology and signaling

CBS-P-01

Comparative characterization of the putative serpentine receptors SR10 and SR12 in the malaria parasite *Plasmodium falciparum*

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Introduction: In eukaryotes, a main signal transduction pathway involves serpentine receptors (SRs), a diverse group of seven-transmembrane proteins that are activated by a variety of ligands. SRs intracellularly couple with trimeric G-proteins and trigger signaling pathways that generally involve the

second messengers cAMP or IP3. During the last years, a number of signaling molecules like cGMP and IP3 have been identified in *Plasmodium falciparum* that are involved in the induction of gametogenesis. However, despite the fact that the *P. falciparum* genomes encodes several putative SRs, to date there is no evidence for the presence of trimeric G proteins, challenging the existence of a G-protein-mediated signal transduction pathway.
Objectives: We aim to analyze the expression of the putative receptors SR10 and SR12 (gene-IDs: PF3D7_1215900 and PF3D7_0422800, respectively) in the blood and transmission stages of *P. falciparum* and to decipher their functions during gametogenesis.
Materials and Methods: Transcript analyses were conducted via semi-quantitative RT-PCR. SR10 and SR12-specific antisera and parasite lines expressing hemagglutinin-tagged SRs were generated to perform protein expression studies via Western blotting, and confocal microscopy. Further, loss-of-function analyses were employed on parasite lines deficient of either SR10 or SR12.
Results: Transcripts for *sr10* and *sr12* are present in trophozoites, schizonts and mature gametocytes. Similarly, SR10 and SR12 proteins can be detected in the cytosol of the blood and sexual transmission stages. For functional studies, gene-disruptant lines (SR10-KO and SR12-KO) have been generated via single crossover homologous recombination, which are currently being used for phenotypical analysis.
Conclusion: SR10 and SR12 are expressed in the asexual blood stages as well as in gametocytes of *P. falciparum*. Due to their intracellular localization, they are not expected to act as classical cell membrane-associated SRs.

CBS-P-02
***Plasmodium falciparum* ubiquitin transferase, a novel putative quinine resistance marker**

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Ubiquitination is a post-translational modification that regulates many essential cellular processes such as protein degradation, cell cycle progression, transcriptional regulation or protein trafficking. The ubiquitin system includes a cascade of enzymes including ubiquitin activating enzymes (E1s), ubiquitin conjugating enzymes (E2s) and ubiquitin ligases (E3s) that label substrate protein with a ubiquitin moiety.
A HECT ubiquitin ligase, called *Plasmodium falciparum* ubiquitin transferase (PfUT), belongs to the family of E3-type ubiquitin ligases. PfUT localizes to the parasites's ER/Golgi complex but its functional role still needs to be validated. Interestingly, it has previously been shown that *pfut* alters quinine and quinidine responsiveness, hence it is also a novel candidate gene for multifactorial resistance to quinine. How PfUT affects quinine responses is, however, currently unclear.
The biological function of PfUT in *P. falciparum* blood stages and its role in resistance to quinine are being investigated. One way of understanding the functional role of PfUT is to generate a conditional knock-down of *pfut* gene. CRISPR-Cas9 system has been used to incorporate the glucosamine inducible *glmS* ribozyme within the 3' untranslated region of PfUT, leading to downregulation of the target protein upon glucosamine addition.
PfUT knockdown line has been generated in *P. falciparum* 3D7 strain. Interestingly, already insertion of *glmS* sequence to the parasite's genome (without glucosamine addition) impaired the parasite growth and increased its susceptibility to quinine, not affecting number of merozoites generated per schizont nor parasite morphology. Glucosamine treatment did not lead to further decline in parasite growth

and it did not affect the morphology. Further studies regarding validation of PfUT's association with resistance to quinine and the identification of its biological targets are currently ongoing.

CBS-P-03
Characterization of adenylate cyclases in *Toxoplasma gondii*

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Cyclic AMP is an essential and ubiquitous regulator of diverse cellular functions across the tree of life including in microbial pathogens. Our previous work has suggested a role of cAMP in host-cell invasion by the tachyzoite stage of *Toxoplasma gondii*. Adenylate cyclase (AC) is the first enzyme that produces cAMP to initiate the signaling cascade; however, there is not much known about these proteins in *T. gondii*. Our current work has identified four putative ACs in the parasite. ACα1, ACα2 and ACα3 genes encode for large proteins of about 900 amino acids and contain one cyclase domain, whereas ACβ is even longer (2040 aa) and comprises two cyclase domains. ACα2 under the control of native elements localizes in the parasite cytosol, whereas ACβ is expressed in the rhoptry neck region. ACα1 and ACα3 are apparently not expressed in the tachyzoite stage. We were able to delete genes for all enzymes using CRISPR/Cas9-mediated homologous recombination, indicating their nonessential and possibly redundant roles during the lytic cycle of tachyzoites. The ACβ knockout showed a growth defect, and by contrast ACα1 mutant exhibited a growth advantage. Genetic ablation of ACα2 and ACα3 did not exert a noticeable phenotype in tachyzoites. Future work aims to determine the underlying reason for these growth defects and elucidate the catalytic activity of these enzymes.

CBS-P-04
A novel Golgi-dwelling phosphatidylinositol synthase is essential for the lytic cycle of *Toxoplasma gondii*

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Phosphatidylinositol (PtdIns) is not only an integral component of biological membranes, but also a precursor for several key signaling mediators (phosphoinositides, glycosylphosphatidylinositol). PtdIns and its derivatives have been implicated in regulating the asexual reproduction of the prevalent parasitic protist, *T. gondii*; however the mechanism, location and physiological importance of PtdIns biogenesis remain to be understood. Here, we demonstrate the presence of a novel PtdIns synthase (PIS) expressed in the fast-replicating acute (tachyzoite) stage of *T.gondii*, which resides exclusively in the Golgi network. *Tg*PIS encodes for a functional enzyme with a catalytically essential CDP-alcohol phosphotransferase motif. The protein also harbors a prolonged N-terminus, which is dispensable for the enzymatic activity as well as localization in Golgi. Unable to synthesize myo-inositol, the parasite imports it from milieu and co-utilizes with CDP-diacylglycerol to produce PtdIns in a time and concentration-dependent manner. Conversely tachyzoites are unable to salvage PtdIns from their environment, and *Tg*PIS is refractory to genetic deletion, suggesting a critical role of *de novo* lipid synthesis. Likewise, the conditional mutagenesis of *Tg*PIS using two independent approaches

(Cre/LoxP and mAID system) confirmed its essential requirement for the lytic cycle of *T. gondii*. Collectively, the data show a strict dependence of the parasite on autonomous PtdIns biogenesis, which can be exploited for therapeutic purposes.

CBS-P-05
BioID-based proximity screening for interaction partners of *Trypanosoma brucei protein* kinase A

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The unicellular parasite *Trypanosoma brucei* is exposed to rapidly changing environments during its development in the mammalian host and the the tsetse fly vector. The unconventional protein kinase A (PKA) of *T. brucei* is activated by key environmental changes that occur during the host to vector transition, including temperature and pH fluctuations. Three catalytic subunit isoforms (PKAC1-PKAC3) seem to mediate distinct and specific signals in this context. The putative signaling pathway(s) upstream of PKA that perceive the environmental signals are completely unknown. To identify components of PKA signaling in this parasite, we performed a BioID-based proximity screen in procyclic trypanosomes for potential interaction partners (direct and transient) of the PKAC subunits. Hits might be involved in activation, localization, transport of the kinase or be downstream targets. After *in vivo* tagging of PKAC and PKAR subunits as BirA*- fusions and biotinylation, the potential interaction partners were pulled down using streptavidin beads and identified by mass spectrometry. Filtering and comparing the datasets will be presented as well as further validation of candidates by localization studies.

CBS-P-06
Development of a screening assay for regulators of protein kinase A signaling in *Trypanosoma brucei*

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The unicellular eukaryotic parasite *Trypanosoma brucei* is the causative agent of Human African Sleeping Sickness, a lethal disease, and Nagana in domestic animals with severe impact on the health and economy of sub-Saharan countries. To date, no complete signal pathways has been described, which is mostly due to the lack of evolutionary conservation of many signaling proteins. The cAMP-dependent Protein kinase PKA is an important component of many eukaryotic signal transduction pathways. Surprisingly, the *T. brucei* PKA orthologue is not activated by cAMP, while otherwise exhibiting some characteristic PKA features such as phosphorylation of canonical PKA substrates and inhibition by the PKA-specific inhibitor peptide PKI. We have identified environmental conditions such as cold-shock that activate PKA *in vivo*, most likely acting via an intracellular upstream signaling pathway. Yet, the components of this pathway are completely unknown. In order to systematically identify PKA regulators in *T. brucei*, we aim to conduct a genome-scale RNAi screen using PKA subunit interaction and thus activation as read-out. Here, we compare three tractable signal-and-response

methods NanoBiT, NanoBRET and FRET, all based on molecular proximity. Our PKA NanoBiT assay shows a wide dynamic range upon treatment with specific activators or inhibitors of PKA, providing a sensitive method for monitoring dynamic PKA activity *in vivo*. NanoBRET and FRET have a relatively narrow dynamic range, but their compatibility with flow cytometry may enable a FACS-based high-throughput genome-wide RNAi screen for PKA regulators. Alternative formats of this screen will be discussed.

CBS-P-07
Optogenetic regulation of cGMP signaling in *Toxoplasma gondii*

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Cyclic GMP and calcium signaling play major roles in regulating the lytic cycle of the intracellular parasite *T. gondii*, which starts with the invasion of the host cell and ends with the egress by lysis. However, there is no direct evidence as yet demonstrating a connection between the induction of cGMP signaling and the lytic cycle of the parasite. We have utilized optogenetic approach involving a light-activated rhodopsin-guanylate cyclase (RhoGC) isolated from an aquatic fungus *Blastocladiella emersonii* (Avelar *et al.* 2014) to modulate the cGMP signaling in the tachyzoite stage of *T. gondii*. An epitope-tagged RhoGC (RhoGC-3xHA) was expressed under the regulatory elements of dihydrofolate reductase/thymidylate synthase (DHFR/TS) targeted at the uracil phosphoribosyltransferase (UPRT) locus. Ectopic expression of RhoGC is not toxic to the parasite in routine cultures. We show that RhoGC-3xHA localizes in the cytomembranes of the parasite. The transgenic line was illuminated by green light (522 nm) to determine the effect of the activation of cGMP signaling on the motility-dependent invasion and egress events. The gliding motility was increased by about 3-fold in the light-stimulated strain when compared to the dark cultures. Consistently, optogenetic strain showed a 2-fold higher invasion efficiency and rapid egress within minutes. Having an optogenetic strain now enables us to identify downstream mediators of the cGMP signaling by phosphoproteomic analysis. Likewise, the co-expression of RhoGC with genetically-encoded Ca+ sensor, GCaMP6s, shall reveal the relationship between cGMP and Ca2+ signaling during the asexual reproduction of *T. gondii*.

CBS-P-08
Optogenetic induction of cAMP signaling in *T. gondii*

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Light-inducible adenylate cyclases represent a novel method to investigate cAMP signaling in intracellular pathogens. The proof-of-principle implementation of such an optogenetic system recently indicated a role for cAMP in invasion and bradyzoite formation in *Toxoplasma gondii* (*1*). The signaling cascade however is yet to be elucidated.
Building on our previous work, we aimed to generate a transgenic strain of *T. gondii* expressing an improved version of the *Beggiatoa* photo-regulatable adenylate cyclase (bPAC) with a reduced dark activity (bPAC-S27A). This parasite strain will allow further exploration of cAMP signaling in *T. gondii*.

The tachyzoite stage of *T. gondii* was cultured in human fibroblasts. Parasites were transfected with a construct expressing bPAC-S27A under the control of GRA1 elements targeted at the UPRT locus. Growth was assessed by plaque assay. Expression was confirmed by Western blot and immunofluorescent analysis, and cAMP levels were determined using commercial ELISA kits.
Ectopically expressed bPAC-S27A localized to the parasite cytosol. The expression was not toxic to the parasites in routine culture. Measurement of cAMP indicated only a mild dark activity of bPAC-S27A (2-fold increase compared to the parental strain). Exposure to blue light for 2 minutes led to an 8-fold increase in cAMP levels, confirming expression of a functional photo-inducible adenylate cyclase in tachyzoites. The optically induced cAMP was rapidly degraded to the basal level, indicating a reversible nature of the system.
We have established a light-regulatable system for studying cAMP signaling in *T. gondii*. Notably, by reducing the dark activity, we have improved the dynamic range and eliminated the need for chemically-regulated conditional expression. Rapid degradation kinetics facilitate a transient induction of spatially restricted cAMP pulses in a reversible manner. We now plan to use this transgenic strain to identify the functions and mediators of cAMP signaling in *T. gondii*.

1. A. Hartmann *et al.*, Optogenetic modulation of an adenylate cyclase in Toxoplasma gondii demonstrates a requirement of the parasite cAMP for host-cell invasion and stage differentiation. *The Journal of biological chemistry* **288**, 13705-13717 (2013).

Diagnosis

DIA-P-01

Molecular Detection of *Theileria annulata* in Cattle Suffering from Respiratory Affections

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Abstract
Introduction: Bovine Theileriosis is one of the most destructive obstacles for the livestock production in Egypt. Respiratory complications are the main cause of death in the untreated cases **(Al–Gaabary, 1991, 1995; AL-Hosary, 2013).**
Objective: This study was carried out to detect *Theileria annulata* infection in cattle suffering from respiratory manifestation without any clinical signs of theileriosis.
Materials and Methods: The current study was conducted on 100 cattle all of them suffered from respiratory affections. Blood samples were collected and examined from each animal. Giemsa's stained blood smears as one of the conventional method of diagnosis as well as molecular method (Tams-1 target based Polymerase Chain Reaction) were used in order to confirm the infection of *T.annulata (***Coles, 1986; Kirvar, et al., 2000).**
Result: The stained blood smears confirmed the infection in (6%) while the PCR using Tams-1 specific primers of *Theileria annulata* confirmed the infection in four samples (17%) positive.
Conclusion: The obtained results concluded that *T.annulata* infection is one of the main predisposing factors for respiratory affections in cattle.
Key words: Cattle, Tick, *Theileria annulata* , Blood smear, PCR

DIA-P-02
Development of an *Bartonella henselae* specific Human IgG ELISA

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Introduction: *Bartonella henselae* causes cat scratch disease (CSD), an often self-limiting lymphadenitis in immunocompetent patients, and several other clinical entities. While cats are the natural reservoir for *B. henselae*, the pathogen is transmitted by cats, cat fleas and eventually by other arthropods. The clinical symptoms underlying CSD might be similar to those being suspicious for malignant tumors. Thus, an easy and reliable test for *B. henselae* infections is highly desirable.
Objective: The aim of this study is to design an ELISA for detection of *B. henselae* to improve the shortcomings of the currently used immunofluorescent test (IFT), e.g. objective and reproducible results and less hands-on time.
Material and Methods: Test development is based on different *B. henselae* strains and quality assured patient sera [(a) sera positively tested for anti *B. henselae* antibodies via IFT, (b) patients with typical symptoms, (c) patients with PCR-based infection diagnosis]. Antigens were separated by ion exchange chromatography and fractions examined in lineblots. Potential fractions were optimized for ELISA.
Results: Patients with *B. henselae* infections show different patterns of antibody expression in western blots. Thus, there is obviously no universally usable antigen for diagnosis detectable. Crude antigen preparations (liquid grown or with cell culture) are not working reliably as they do not react with numerous patient sera. However, our tests show that there are certain protein fractions from *B. henselae* which react reliably and results from lineblots were successfully transferred to an ELISA-format with sufficient sensitivity.
Conclusion: We show a strategy for antigen testing and selection from *B. henselae* protein preparations for ELISA-based serology. Further processing of antigens is under investigation so that in future an ELISA for *B. henselae* is possible.
Funding: This study is financed by the state Hesse within the LOEWE III project.

DIA-P-03
A novel Loop-mediated isothermal amplification (LAMP) assay for the detection of *Rickettsiae* belong to the spotted fever group and typhus group

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Question: The loop-mediated isothermal amplification (LAMP) is currently applied to detect several zoonotic pathogens including Rickettsiae belonging to the Spotted fever (SFG) and Typhus groups (TG). However the previous published assay, targeting OmpB gene, showed a poorly specificity about several pathogenic rickettsiae. Herein we present a novel and more specific LAMP assay for the detection of Rickettsial pathogens.
Methods: To develop the LAMP assay, 4 novel primers, FIP (5"-GAGAACCAAGTAATGCGCCGGGCGGTATGAATAAACAAGG-3"), BIP (5"-

AATTCGGTAAGGGCAAAGGACCACCGATTTGTCCACCAA-3"), F3 (5"-TGTTACAAGCCTGTAACGG-3") and B3 (5"-TCCTGTTCATCCATACCTG -3") were designed using Primer Explorer Software (http://primerexplorer.jp/e/). The primers were based on the 17kDa gene which encodes an antigen protein of R.rickettsii. The LAMP reaction mixture (final volume, 25 µL) contained the following: 15 µL of isothermal amplification buffer, 4,5 µL nuclease free water, 1.6 µM each of the FIP and BIP primers, 0.4 µM each of the F3 and B3 primers and 5 µL of extracted DNA. The reaction mixture was incubated in a RealTime PCR instrument at 65°C for 50 min. The specificity of the LAMP assay was evaluated by testing 40 different microbes DNAs. To test analytic sensitivity, 10-fold serial dilutions of R. conorii DNA from 8 ng/µl to 8 fg/µl were subjected to LAMP.
Results: LAMP assays of genomic DNA from all the microorganisms tested were negative. The LAMP assay identified all the positive samples with a detection limit quantified to 8 pg of R. conorii DNA/µL. The total time necessary for the LAMP assay, which included amplification and detection was about 50 min.
Conclusions: Our novel Pan-Rickettsia assay resulted to be efficient, specific and sensitive to reveal DNA of pathogenic Rickettsiae belonging to SFG and TG agents. This alternative molecular technique wish be helpful for a rapid diagnosis of this important zoonotic agents**.**

DIA-P-04
Detection of Lyme Borrelia by Loop-mediated isothermal amplification (LAMP): an in-field experience

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Question: The loop-mediated isothermal amplification (LAMP) technique is appreciate for its rapidity, easiness of use and low cost; furthermore it can be considered as a precious in-field molecular technique. We report the results related to the molecular detection of *Borrelia burgdorferi* sensu-lato in questing ticks by LAMP.
Methods: The experiment was carried out in June, 2017 in a wooded area previously positive checked for Lyme disease agents. Questing ticks were collected by using the flagging method, specimens were stored in ethanol 70% and then identified at species level with the aid of a portable stereomicroscope. A sterile pestle and mortar had to be used to bust the tick prior to use a rapid DNA extraction kit. Five µL of the dilution might be directly used as a template in LAMP reactions. The assays were set up by using the Isothemal Master Mix, which contained a DNA binding fluorescent dye. An aliquot of DNA that was found to be positive for *B. afzelii* in our previous investigation was used as a positive control. Reactions were simultaneously run in the portable instrument Genie III and results were visualized in real time. In order to determine the species of *B. burgdorferi* sl., the DNA extracted in field was used in laboratory the next day as templates for PCR targeting *groEL* gene. The gathered amplicon was purified and sequenced.
Results: A total of five nymphes of *Ixodes ricinus* were collected and identified, then used for DNA extraction procedures. Out of the 5 tested ticks, 1 resulted positive for *B. burgdorferi* s.l. by LAMP. The nucleotide sequence of the amplicon was 100% identical to the corresponding portion of the *groEL* gene of *B. garinii.*
Conclusion: We described the one-day campaign aimed to the detection of Lyme borrelia directly on the spot by LAMP approach. This unconventional technique is destined to become a concrete strategy that can provide useful and immediate information in a wide range of vector-borne diseases.

DIA-P-05

A quantitative real-time Polymerase Chain Reaction for the specific detection of *Hammondia hammondi* and its differentiation from *Toxoplasma gondii*

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Introduction: *Hammondia hammondi* and *Toxoplasma gondii* are closely related and morphologically very similar protozoan parasites, but only *T. gondii* is an important zoonotic pathogen. Both use felids as definitive hosts. In *T. gondii*, detection of a 529 bp repeat is of utmost diagnostic importance and we have identified a similar repetitive region in the *H. hammondi* genome.
Material and Methods: Based on reported sequences, primers and probes were selected in silico and together with published primers, optimal primer/probe combinations were explored. Analytical sensitivity was tested using serial dilutions of oocyst DNA. Analytical specificity was confirmed by testing DNA samples from related parasitic species.
Results: Finally, 8 forward and 6 reverse primers were tested in varying combinations. A primer set consisting of a published (Hham34F) and a new primer (Hham157R) revealed optimal results. For this combination, 3 potentially suitable dual-labelled probes were selected. Optimal results were obtained with the probe Hham110P and the primer/probe combination further validated. In addition to excellent analytic specificity, the assay revealed a diagnostic sensitivity of genome equivalents of less than 1 oocyst.
Discussion: The use of the 529 p repeat of *H. hammondi* is ideal for the establishment of a quantitative real-time PCR assay, since this repeat exists probably 200-300–times in the genome of a single organism of *H. hammondi*, similar to the abundance of its counterpart in *T. gondii*. Although we had excellent sequence data, only a single set of the primers predicted in silico yielded sufficient amplification. The identification of a suitable probe was also difficult. This is in accord with our previous observations on the variability in the 529 bp repetitive sequences of *H. hammondi*.
Conclusions: We developed a real-time PCR for the specific detection of *H. hammondi*, which may be used as a novel tool for epidemiological and cell-biological studies.

DIA-P-06

Prevalence of Schistosoma mansoni, Soil-Transmitted Helminths and Intestinal Protozoa - Baseline data of the Ijinga Island Schistosomiasis Elimination Pilot Study, Northwestern Tanzania

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Introduction: Infections caused by S. mansoni, soil transmitted helminths (STHs) and intestinal protozoa (IP) remain highly prevalent at the shores of Lake Victoria. The pilot study on Ijinga Island, Northwest Tanzania, aims to eliminate schistosomiasis, IP and STH infections through a multidisciplinary approach including treatment, health education, WASH activities and snail control.

Objective: The objective of the study was to gather baseline data to monitor the effect and sustainability of elimination strategies.
Methods: Stool samples of 936 study participants were examined by Kato Katz (KK) technique (2 slides). 307 Samples were randomly selected, processed by SAF method and examined microscopically. Urine samples were screened for haemoglobin, positive samples underwent urine filtration.
Results: Using KK the prevalence of S. mansoni was 67.8 %, whereas SAF technique showed 62.5 %. 58.7 % of the positive found had light (1-99 eggs per gram), 27.4 % moderate (200-399 epg) and 13.9 % severe infections (>400 epg). 83.7 % of school aged children (SAC), 60.7 % of preschool aged children (PSAC) and 55.3 % of adults were found positive for S. mansoni. No S. haematobium was found.
Microscopy after SAF processing showed 19.2 % infected with Hookworm and 6.5 % with S. stercoralis. The prevalence of G. intestinalis was 14 %, of E. histolytica/dispar 26.4 %. 81.4 % carried at least one non-pathogenic protozoa.
Conclusion: The highest rate of infection with S. mansoni was found in SAC followed by PSAC. The majority of all participants had light or moderate infections. SAF method proved less sensitive to detect S. mansoni than KK. The prevalence of G. intestinalis and E. histolytica/dispar was also high making them potential biomarkers for the effect of WASH activities. S. mansoni, STH and IP infections are highly prevalent and remain a serious health problem on Ijinga Island. Adequate treatment as well as health education and infrastructural improvement are urgently needed.

DIA-P-07
A prospective evaluation of an automated detection of malaria parasites using the CellsCheck ™ compared to expert microscopy

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Questions: The microscopic detection of malaria parasites requires expertise and is time consuming, in particular for low parasite densities. We prospectively evaluated the performance of an automated detection system using the CellsCheck ™ platform compared to expert microscopy over a period of 4 months in a routine setting. The following criteria were used: sensitivity and specificity compared to expert microscopy, handling and ease of use, duration of testing (for individual and multiple samples).
Methods: We consecutively enrolled all patients presenting at our travel with a history suggestive of malaria. Malaria testing was ordered by the attending physician (a specialist for tropical medicine) based on the history and clinical evaluation of the patient. Microscopy was performed on thick and thin films. Testing was performed sequentially, in most cases by the same technician, so that results for microscopy were reported before the results of the CellsCheck™ were available.
Results: A total of 138 consecutive specimens were examined for malaria by microscopy of which 16 (11%) were positive for malaria (12 P. falciparum, 2 P. vivax, 2 P. ovale). CellsCheck ™ results were available for 127 (93%) of these. In two cases (both slide negative) the samples could not be processed by the system ("rejected"). The remaining 9 cases were not done due to time constraints (at the weekend). There was full concordance (100%) for both the positive and negative samples, and there were no discrepancies in identification of parasite species. The system is easy to use and can process up to 5 samples simultaneously. Processing time for individual specimens is shorter than microscopy.

Discussion: The CellsCheck ™ platform is a promising tool for the diagnosis of malaria. Results are comparable to malaria microscopy. The system is easy to use. The advantages and limitations of the system are discussed.

DIA-P-08
A modification of the Harada-Mori-Culture for the detection of nematode larvae in fecal samples

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Introduction: Beside the Baermann test and the Koga agar plate method the Harada-Mori-Culture is one of the classical parasitological techniques to demonstrate *Strongyloides* and hookworm larvae in fecal samples. Despite the trend to molecular techniques we tested a modification of the Harada Mori-Culture in a proof of concept study. The modified technique allows using a larger sample volume and direct detection of larvae with an inverted microscope.
Methods: The conical end of 50 ml polypropylene centrifuge tubes was cut off and a 10 mm microscopic cover glass was glued to the end of the tube using a special primer and cyanoacrylate adhesive. This allowed the use of an inverted microscope for direct examination. A stripe of stiff chromatography paper instead of filter paper was inserted into the partially water-filled tube and 0,5-1g of fresh faeces applied. 278 fecal samples from an outpatient HIV clinic at a reference hospital in Tanzania were examined. Incubation was at ambient temperature (28°C). The tubes were examined every 24h for 6 days.
Results: Up to day 3 only 1/278 samples was positive. On the 6th day 13/259 (5%) tubes yielded nematode larvae but 19 (6,8%) tubes could not be assessed anymore due to fecal sediments covering the glass bottom of the test tubes. Differentiation of hookworm and *Strongyloides* larvae proved to be difficult requiring experience.
Conclusion: The advantage of the modified Harada-Mori-Culture is the direct observation of nematode larvae avoiding the transfer from the culture tube (risk of loss of larvae) for microscopy. Little non-reusable material (stripes of chromatography paper) is required making the method inexpensive. In settings where PCR, the technique with the reported highest specificity and sensitivity, is not available, this technique might be a considerable alternative. Comparative studies are required to assess sensitivity and specificity.

DIA-P-09

Performance of a malaria rapid diagnostic test, CareStart™ Malaria HRP2/pLDH (pf), and diversity of *P. falciparum* histidine-rich proteins 2 and 3 in Busia County, Western Kenya

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Background: Rapid diagnostic tests (RDT) are valuable tools in the diagnosis of *Plasmodium falciparum* infection. RDTs support prudent and timely use of antimalaria drugs, particularly if reliable microscopy is not available. However, the performance and reliability of these tests vary between and within geographical regions. We evaluated the performance of the CareStart™ Malaria HRP2/pLDH (pf) RDT, a *Plasmodium falciparum*-specific RDT, using Giemsa microscopy as the gold standard and assessed the *P. falciparum* amino acid sequence diversity of the histidine-rich proteins 2 and 3 (PfHRP2 and PfHRP3) in a malaria endemic setting in Busia County, western Kenya.
Methods and Results: A total of 192 malaria suspected cases were included and finger-prick blood was obtained. By microscopy, 76 (39.6%) cases were detected, all of them caused by *P. falciparum*. The RDT CareStart™ HRP2/pLDH (pf) detected 101 (52.6%) cases, while nested PCR was able to identify 80 (41.7%) cases. When microscopy and nested PCR were used as reference methods, the sensitivity was 94.7% and 93.8% while the specificity was 75% and 76.8%, respectively. High negative (94%) and low positive (74%) predictive values were observed. No case of *pfhrp2* and/or *pfhrp3* deletion was identified among the *P. falciparum* isolates. A high degree of PfHRP2 amino acid sequence diversity was observed. In contrast, PfHRP3 amino acid sequences were less diverse. Eleven PfHRP2 and 9 PfHRP3 amino acid repeat variants were identified.
Conclusion: The CareStart™ HRP2/pLDH (pf) RDT is suitable for falciparum malaria diagnosis in Busia County in spite of high PfHRP2 diversity. It can, therefore, reliably be used where microscopy is not available. Regular studies should be undertaken to evaluate the suitability of PfHRP2-based RDTs in other malaria settings in Kenya, especially in low transmission areas where PfHRP2 and 3 antigen diversity may have a profound impact on the test performance. Causes of RDT false negative results such as *pfhrp2/3* gene deletion should also be investigated.

DIA-P-10
Wet mount microscopy, mini-FLOTAC and PCR for the diagnosis of *Ascaris lumbricoides*

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In many resource-limited areas, single wet mount microscopy is the usual tool for the diagnosis of soil-transmitted helminths in stool samples. Mini-FLOTAC is another simple low-cost method, while PCR assays presumably show the highest sensitivity. The clinical relevance of infections additionally detected by the more advanced techniques is not well established. In southern highland Rwanda, an area of predominant *Ascaris lumbricoides* infection, stool samples from 845 schoolchildren were examined for this parasite by wet mount microscopy, Mini-FLOTAC, and PCR.
Based on wet mount microscopy, Mini-FLOTAC and PCR, the prevalence of *A. lumbricoides* infection was 25%, 32%, and 37%, respectively. Agreement was moderate for wet mount microscopy and Mini-FLOTAC or PCR, and good for Mini-FLOTAC and PCR. Taking *A. lumbricoides* diagnosed by any of the three methods as reference, the sensitivities (and 95% CIs) of wet mount microscopy, Mini-FLOTAC, and PCR were 56.4% (51.3-61.4), 71.9% (67.1-76.3), and 81.9% (77.6-85.6), respectively. Clinical manifestation was associated with *A. lumbricoides* infections diagnosed by Mini-FLOTAC but not by wet mount. For infections detected by PCR exclusively, hardly an association with clinical symptoms was seen.
PCR had the highest sensitivity. In contrast, almost half of the *A. lumbricoides* infections actually present were missed by wet mount microscopy. Mini-FLOTAC showed interim sensitivity including otherwise undetected, clinically relevant infections. These findings argue for the extended use of Mini-FLOTAC in patient management in endemic regions and for the application of PCR in assessing actual prevalence and epidemiology.

Drugs and drug development

DDD-P-01
Medicinal plants with promising antileishmanial activity in Iran: asystematic review and meta-analysis

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Background: Leishmaniasis is a major public health problem worldwide. The aim of the present study was to investigate medicinal plants with anti-Leishmania activity which used in Iran.

Methods: Data were systematically gathered from five English databases including Ebsco, Science Direct, PubMed, Google Scholar and Scopus, four Persian databases including Magiran, Iran doc, Iran medex and the Scientific Information Database (SID) from 1999 to April 2015. Information obtained included plant family, extraction method, concentrations of extracts, animal models and parasite strains.
Results: A total of 68 articles including 188 experiments (140 in vitro and 48 in vivo) between 1999 and 2015, met our eligibility criteria. Thoroughly, 98 types of plants were examined against three genera of Leishmania spp. For the heterogeneity study conducted, it was showed that there was a great deal of variation among studies. Based on random effect, meta-analysis pooled mean of IC50 was obtained 456.64 (95% CI: 396.15, 517.12).
Conclusion: The most Iranian plants used as anti-leishmanial activity were Artemisia species, Allium sativum, Achilleamille folium, Peganum harmala and Thymus vulgaris. The present systematic and metaanalysis review provide valuable information about natural products with anti-Leishmania activity, which would be examined in the future experimental and clinical trials and herbal combination therapy.

DDD-P-02
Studying the Chemical Composition in Vitro Activity of *Cinnamomum zeylanicum* and *Eugenia caryophyllata* Essential Oils on *Leishmania major*

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Leishmaniasis is an important protozoan disease, which is still a widespread disease. In addition, avoiding drug resistance is extremely important, and herbal medicines may play a major role in reducing resistance to chemical drugs. This fact is considered by the World Health Organization. The main objective of this study was to assess the effects of *Cinnamomumzeylanicum (C. zeylanicum*) and *Eugenia caryophyllata*essential oilson *Leishmania major* under in vitro conditions. In the present study, the concentrations of 31.25, 62.50, 125, 250, 500, 1000 µg/mL of *C. zeylanicum*and *E. caryophyllata* extracts were assayed in vitro by MTT (3-(4,5-dimethylthiazol- 2yl)-2,5-diphenyltetrazolium bromide) against the promastigote forms of leishmania *major*. The results indicated that each medical herb was compared with other medical herbs and also with the results of the control groups. The results associated with promastigote assays showed that when the dose of both medical herbs increased, the promastigotes populations were reduced in comparison with the control group.

DDD-P-03
Evaluating the Effect of Protein Kinase Inhibitor (imatinib) in comparison to Praziquantel on *Schistosoma mansoni* Infection

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Introduction: Schistosomiasis affects about 210 million people worldwide, treatment relies basically on the use of Praziquantel (PZQ); However, massive administration of PZQ in endemic areas and the

ineffectiveness of the drug towards immature stages have raised critical concerns. Protein tyrosine kinases (PTKs) are identified nowadays as potential targets against schistosomes through their essential action on the development and metabolism of the parasite.

Aim of Work: The aim of the present study is to assess the anti-schistosomal activity of Protein tyrosine kinase inhibitors (Imatinib) as therapeutic agent against juvenile and adult *S. mansoni* infection in mice in comparison to praziquantel.

Method: A batch of 120 male Swiss albino mice of CDI strain were used, divided into three main groups (control group, drug group of acute infection, drug group of chronic infection) each group divided into different sub groups. The anti-schistosomal activity of the protein tyrosine kinase inhibitor (Imatinab) in comparison to praziquantel in both acute and chronic *S. mansoni* infection was assessed regarding different parameters; parasitological (worm burden, oogram pattern), histopathological (liver sections stained with Hematoxylin and Eosin and Masson"s trichrome stain) as well as immunohistochemistry (effect of Imatinib on the expression of TGF-β 1 in the cytoplasm of hepatocytes).

Results: As regards the examined Parameters; Imatinib showed significant reduction in comparison to Praziquentel espicially when combined with it. **Conclusion:** Protein tyrosine kinase inhibitor (Imatinab) especially when combined with praziquantel ; showed promising anti-schistosomal and anti-fibrotic action.

keywords: *Schistosoma mansoni*, Imatinib, Praziquantel, Protein kinases.

Figure 1

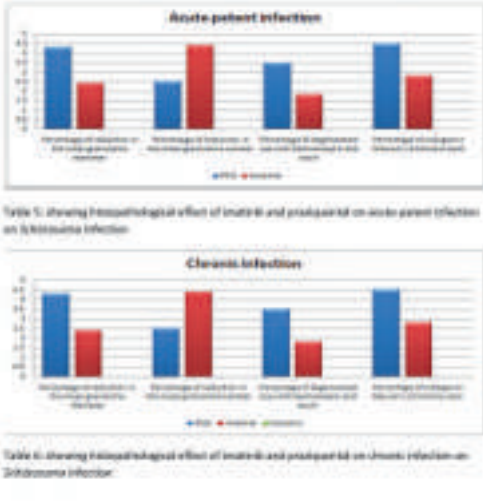
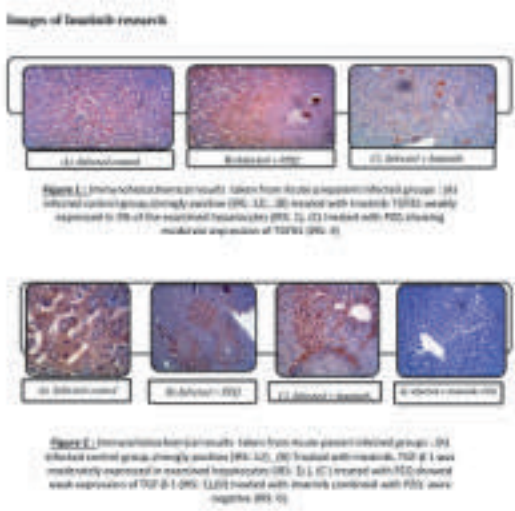


Figure 2



DDD-P-04

Proteome Analysis of Excretory-Secretory Products of *F. hepatica* in the Presence or Absence of Triclabendazole (Anthelmintic Drug) Using Two-Dimensional Gel Electrophoresis

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Introduction: *Fasciola hepatica* is Platyhelminthes trematode that causes fascioliasis. The parasite migrates in liver parenchyma by secreting various excretory-secretory (ES) protein. Excretory-secretory proteins could be considered as a marker in the interactions between parasite and host and could be a target in diagnosis, treatment (drug and drug development), and even candidates for vaccine production. Triclabendazole (TCBZ) is the drug of choice for treatment of fascioliasis.

Objectives: The aim of this study was to compare the protein amount and protein spots of ES products of *Fasciola hepatica* using two-dimensional gel electrophoresis method in the presence or absence of TCBZ.

Materials & Methods: *F. hepatica* parasites were collected from infected cattle livers, divided in two groups and cultivated in RPMI 1640 medium. The first group was treated with TCBZ and second group considered as control. The ES products of each group were separated and total protein determined by the Bradford method. To provide proteome spots, the ES proteins were precipitated and Two-

dimensional gel electrophoresis (2-DE) prepared. Protein amounts of the two groups were compared using the statistical t-test and protein spots from 2-DE in the test and control groups were also statistically analyzed.
Results: The t-test showed a significant increase of total proteins in the treated group ($P<0.5$). The protein spot count in the control group was less than the test group, however, statistically not significant ($p>0.05$). Protein spots MW 36.7 pH 5.34, MW 28.2 pH 5.36, MW 36.5 pH 5.8 and MW 36.6 pH 6.26 were appeared and identified in test groups. These proteins belong mostly to protease enzymes.
Conclusion: TCBZ affect the quantity and probably the quality of parasite proteome. It seems that, these results can be considered to determine the proteins which are produced in an ES material after using drug.

DDD-P-05
Structure-Based Design and Synthesis of Dual Targeting Inhibitors for the Treatment of Parasitic Infections

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Human parasitic infections are among the major and most serious health problems, especially in the underdeveloped regions of the world. Limited number of available drugs, side effects and rapid development of resistance necessitate the discovery of new therapeutics with novel mechanisms of action [1]. In this regard, the "repurposing or piggy back" strategy was found to be very effective in the field of epigenetics, as many epigenetic targets –mainly histone modifying proteins- were found to control gene expression in major human parasites [1-6].
We previously reported a series of 3-amidobenzhydroxamates as novel inhibitors of schistosomal HDAC8 for the treatment of schistosomiasis [7]. In addition, several HDAC inhibitors, previously synthesized in our group, exhibited potent antiparasitic effect against *Plasmodium falciparum* and *Trypanosoma cruzi* (unpublished data). However, the rapidly developed resistance remains always a major concern. In this regard, designing drugs capable of inhibiting multiple targets can be very beneficial to overcome this problem [8, 9]. In the present work, we aimed to synthesize inhibitors that can target not only HDACs of parasites, but also other epigenetic targets such as bromodomains, or metabolic targets such as dihydrofolate reductase (DHFR). General structure of some of these dual inhibitors are shown in the attached figure.
Biological evaluation of the synthesized compounds was carried out in vitro and in cellular systems. Some of the compounds showed potent inhibitory activity, mainly against *Plasmodium falciparum* and *Trypanosoma cruzi.*

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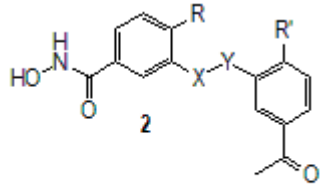
Figure 1



2

X: CO, CH_2, NH; Y: NH, NH, CO
R: CH_3, OCH_3, Cl;, R': CH_3, C_2H_5

DDD-P-06

Structure optimization of 3,4-disubstituted Benzhydroxamates as modulators of epigenetic targets for the treatment of parasitic diseases.

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As part of the A-ParDDise EU project it could be shown that the anti-parasitic epigenome targeting strategy can be used to treat neglected diseases caused by eukaryotic pathogens [1]. Here we report the inhibition of the schistosomial HDAC8 (smHDAC8) as promising approach for the treatment of schistosomiasis. *Schistosoma mansoni* histone deacetylase 8, the most expressed class I HDAC isotype in schistosomes, is a zinc-dependent protein deacetylase that plays an important role in parasite infectivity [2]. Structure guided optimization of smHDAC8 hits [3], based of computational approaches [4] and protein crystallization leads to compounds with high inhibitory activity against schistosomes viability, pairing and reproduction. The most promising inhibitors were profiled for toxicity and pharmacokinetic properties and are produced in enough amount for in vivo studies.

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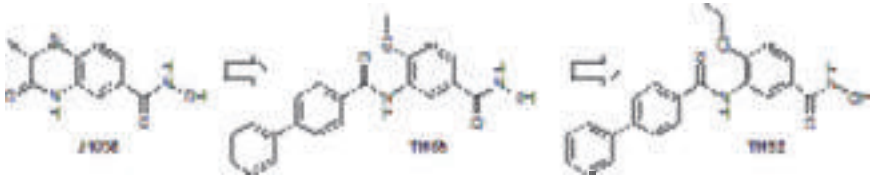
Figure 1



DDD-P-07

Control of intracellular *Leishmania major* infection by treatment with synthetic analogs deduced from an *Entamoeba histolytica* glycolipid

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Immune stimulatory molecules that influence the activation stage of macrophages infected with *Leishmania (L.) major* parasites represent a promising strategy to treat cutaneous leishmaniasis (CL), a disease lacking a satisfying therapy at present. We recently identified an immune stimulatory molecule within the membrane of the parasite *Entamoeba histolytica*, the lipopeptidephosphoglycan (*Eh*LPPG). The glycophosphatidylinositol anchor of this glycolipid interacts with Toll-like receptors of antigen presenting cells thus stimulating a protective immunity.

Objectives: We analyzed the therapeutic efficacy of *Eh*LPPG and synthetic analogs derived thereof using *in vitro* and *in vivo* models for *L. major* infection.

Materials & Methods: Bone marrow-derived macrophages (BMDMs) and the human macrophage cell line (THP1) were infected with *L. major* promastigotes and treated with *Eh*LPPG and its synthetic analogs. The *Leishmania* load was determined using a Taqman PCR and a High Content Screening System. We analyzed the treatment specific immune response using multiplex cytokine bead assay and qPCR. Treatment efficacy of the synthetic analogs *in vivo* was assessed using two murine models for infection.

Results: *L. major* load in infected BMDMs and THP1 cells was significant reduced after treatment with *Eh*LPPG and five out of ten synthetic analogs. Local administration of synthetic analogs reduced the *Leishmania*-induced footpad swelling in the BALB/c model and the ear swelling in the C57BL/6 model for CL, respectively. Cytokines within lysates from *Leishmania* lesions showed a treatment-specific reduction in the Th2 type of immune response.

Conclusion: The identified immunostimulatory molecules so far support and accelerate the clearance of *Leishmania*-induced infections and thus represent promising candidates for new treatment strategies against CL.

DDD-P-08

Establishment of a screening platform for testing compounds against *Schistosoma mansoni in vitro*

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Schistosomiasis (Bilharzia) is caused by schistosome parasites and it is one of the most serious and widespread infectious disease worldwide.
Particularly affected are humans in tropical and subtropical areas. To date, the sole effective available drug for the treatment is Praziquantel (PZQ). However, in field trials a decreased efficacy of PZQ against schistosome parasites was shown, hinting for a gradual resistance formation in the parasites.
Therefore, it is mandatory to find new drugs as alternatives to PZQ. Since the reproduction of the schistosome parasites is only possible using *in vivo* models, screening for interesting compounds is laborious and limited in scale. Thus, the development of an *in vitro* assay may provide a high-throughput-screening platform that could be crucial in selecting potential candidate compounds. At that the same time the need for animal experiments could be diminished.
For this purpose, two exemplary enzymes, one aldehyde dehydrogenase (ALDH) and the Abelson protein-tyrosine kinase 2 (Abl2) of *S. mansoni* were selected as potential drug-targets. These enzymes were shown before to play central roles in the survival of the parasites. Furthermore, the Abl2 and the ALDH are of particular interest because of orthologs of these enzymes exist also in other parasites.
The enzymes will be manufactured using the baculovirus-expression-vector-system (BEVS) and *Spodoptera frugiperda* Sf9 cells. Purification of the proteins will be achieved using affinity (His-tag), and, after proteolytic cleavage, ion exchange, and size exclusion chromatography. The purified enzymes will be integrated into an ATP and NAD(P) assay, respectively.
In order to develop a robust screening platform, the enzymes will be characterized concerning their kinetics and stabilities. For proof of concept, the established test system will be used also for screening of insect-based molecules. Additionally, the platform will be extended to screen for drug candidates using respective enzymes originating from trematodes and other parasites.

DDD-P-09

Antileishmanial activity and *in silico* mechanism of action of fucosterol isolated from the brown seaweed *Sargassum vulgare* C. Agardh

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Leishmaniasis is one of the most serious and most neglected tropical diseases worldwide responsible for nearly 70,000 deaths annually. Combined with limitations such as long-term administration, toxicity, unaffordable cost, available drugs shown a significant decrease in effectiveness in the last few decades. In addition, the emergence of drug-resistant emphasis the need for new drugs. In recent years, there has been a growing interest in the therapeutic use of marine algae as a source of compounds to treat parasitic diseases. In that framework, we screened extract, fractions, and a compound from the marine macroalga *Sargassum vulgare* against promastigotes and intracellular amastigote of cutaneous and visceral leishmaniasis parasites using the MTS colorimetric and the trypanothione reductase-based assays respectively against the promastigotes and intracellular amastigote forms. Cytotoxicity of active extracts on cells was assessed using the resazurin assay. Fractions and isolated compound, fucosterol exhibited strong anti-*Leishmania* activity against the promastigotes forms of *L. major* and *L. donovani* with IC_{50} values of 18.99-107.60µg/mL. We confirmed that active fractions and fucosterol were not toxic to macrophages (RAW 264.7), human normal skin fibroblast cells (NB1RGB) and Chang liver cells at a concentration of 200µg/mL. The *in silico* homology modeling of trypanothione reductase of *L. donovani* and *L. major* and molecular docking of fucosterol were performed in order to elucidate the exact mechanism of binding action of fucosterol. Further pharmacodynamic profiling was carried out to provide details as to pharmacological potency as an antileishmanial agent. The results of the studies indicate that fucosterol might be a promising additive in the combined drug inhibitor of trypanothione reductase. These findings indicate a potential beneficial effect of fucosterol from the brown macroalga *Sargassum vulgare* and justify its *in vivo* evaluation.

DDD-P-10

Synthesis and *in vitro* Testing of Dithiocarbamates as Novel Anthelmintic Inhibitors against Schistosomiasis

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Schistosomiasis, also known as bilharzia, is a chronic parasitic disease infecting more than 250 million people. At least 200,000 dead persons are associated with the disease. Beyond Malaria, it is the

second most important parasitic disease worldwide occurring in over 70 countries in tropical and subtropical regions. The schistosome species are transmitted by contact of water containing free-living larval forms of the parasite (cercariae) originating from intermediate host snails. Due to the lack of a vaccine, the therapy is restricted to a single drug. Praziquantel is the only drug effective against all schistosome species and has been used for more than 40 years. Every year, millions of people are medical attendance with praziquantel. Therefore, the fear of drug resistance encourages the search for novel anthelmintic drugs against schistosomiasis.
Dithiocarbamates were identified as anthelmintic compounds from a screening with disulfiram. Disulfiram was used for the treatment of chronic alcoholism by inhibiting the acetaldehyde dehydrogenase converting acetaldehyde to acetic acid. It was recognized that disulfiram also disintegrates the surface structure of schistosomes, the tegument, leading to the death of the parasites. We synthesized dithiocarbamates instead of dithiocarbamate disulfides. Our dithiocarbamate compounds show significant effects on adult schistosomes *in vitro*. Possibly, this discovery might open a new direction for chemotherapy of human schistosomiasis.

DDD-P-11
Synthesis and biological evaluation of biarylalkylcarboxylic acid derivatives

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Schistosomiasis is a parasitic disease which leads to 200 million infections and 200 000 deaths per year worldwide. The WHO categorises this infection as a neglected tropical disease. Since *Schistosomiasis* is mainly treated solely by Praziquantel, the fear of upcoming resistance is increasing. In the last few years, decreased sensitivity of the parasites towards Praziquantel was observed. This leads to the urge of the development of new antischistosomal agents. [1] Schistosomal aldose reductase (AR) seems to play a crucial role in antioxidant pathways and protection of the worms towards its host"s ROS attack. [2,3] As this is an important factor for the worms" survival in the host, AR is a promising drug target. Testing human AR inhibitors with an biarylalkylcarboxylic acid scaffold showed a promising activity against *Schistosoma mansoni* pairs *in vitro*. [4] Further SAR modifications led to an increased antischistosomal activity and phenotypes such as decreased pairing stability, motility and egg production. Current optimization studies focus on the variation of the carboxylic acid moiety.

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Drug resistance

DRE-P-01

Effect of albendazole and mebendazole on drug-metabolizing enzymes in *Hymenolepis diminuta*

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It is known that tapeworms are capable to metabolize benzimidazole anthelmintic drugs. On the other hand information about effect of these drugs on the activities of drug metabolizing enzymes and antioxidant enzymes in tapeworms is lacking. Therefore, aim of our work was to evaluate if albendazole and mebendazole, representatives of benzimidazole anthelmintic drugs, are capable to influence activities of these enzymes as mainly increased activities of these enzymes can contribute to drug resistance development.
As a model tapeworm for this study rat tapeworm (*Hymenolepis diminuta*) was used. Adult tapeworms isolated directly from the host (rat) were incubated (5 % CO2, 37 °C) for 24 hours with albendazole and mebendazole (1 and 10 µM, in DMSO) in RPMI-1640 medium. Control group was cultivated in drug free RPMI-1640 medium with DMSO. Each group contained 6 tapeworms. After the incubation, subcellular fractions were prepared (microsomal, mitochondrial, and cytosolic) and enzyme activities evaluated (Peroxidase, Catalase, Superoxide dismutase, Acenaphthenol dehydrogenase, Menadione reductase, 4-pyridinecarboxaldehyd reductase, Glutathione S-transferase, Glutathione reductase, UGT-glucuronosyl transferase and UDP-glucosyltransferase).
Results show, that in the group treated by mebendazole enzyme activities from cytosolic fraction were significantly increased (4-pyridinecarboxaldehyd reductase, glutathione S-transferase, superoxide dismutase and peroxidase). In the group treated by Albendazole enzyme activities in cytosolic fraction were also increased (Menadione reductase, Glutathione S-transferase and Superoxide dismutase). Mitochondrial and microsomal fractions don't seem to be significantly influenced in both groups.
Based on our results, we can conclude that *H. diminuta* is capable to increase activities of drug metabolizing enzymes and antioxidant enzymes as a reaction to presence of both benzimidazole drugs. In the group treated by Mebendazole, increased activities of carbonyl-reducing enzymes indicated by increased activity of 4-pyridinecarboxaldehyd reductase can lead to increased production of reduced Mebendazole.

Financial support for this project was provided by Charles University (research project SVV 260 416)

Epidemiology and emerging infections

EPI-P-01

Bartonella spp. in Domestic and Wild Animals and their Ticks in Hesse, Germany – Serology, PCR and Microbiome analysis

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Introduction: Zoonotic agents pertaining to the genus *Bartonella* are usually transmitted via blood-sucking arthropod vectors and cause a constantly increasing number of human and animal diseases.
Aims: In this "*one health*" approach, we screen wild and domestic animals and their ticks from Hesse, Germany for the presence of *Bartonella* spp. or related infections.
Materials and Methods: To date, 81 ticks from 52 dogs, one tick from one cat, 82 ticks from 34 roe deer, one tick from one raccoon and four ticks of four boars from Hesse were screened via PCR. Serum of the cat and dogs was screened for anti-*Bartonella*-antibodies via an indirect immunofluorescence assay and EDTA-blood of wild animals was examined via PCR. Screening for the presence of *Bartonella* spp. in ticks and EDTA-blood was conducted via 16S-23S-ITS- and 16S-rDNA-PCRs. To distinguish the closely related *B. schoenbuchensis* and *B. capreoli,* rpoB-PCR and subsequent detailed sequence analysis was performed.
Results: Three different *Bartonella* spp. were found in total. *B. henselae* was detected in three ticks from two roe deer. *B. capreoli* was detected in the blood of two roe deer and *B. schoenbuchensis* was detected in the blood of seven deer and in three ticks of two deer. Furthermore, a co-infection with *B. schoenbuchensis* and *B. capreoli* was detected in the blood of two deer. One dog was seropositive for anti-*Bartonella*-antibodies. Microbiome analysis of ticks is ongoing and will reveal the prevalence of pathogenic bacteria in those ticks to assess the infection risk for both humans and animals.
Conclusion: The results indicate low *B. henselae* prevalence in dogs and their ticks in Hesse, Germany whereas the ruminant associated *B. capreoli* and *B. schoenbuchensis* (which is suspected to cause deer ked dermatitis) can be found regularly in roe deer and their ticks.

EPI-P-02

Diagnostic validation of a magnetic capture PCR for the copro-diagnosis of *Echinococcus multilocularis* infection in foxes using the Intestinal Scraping Technique as a reference

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Introduction: The Intestinal Scraping Technique (IST) and the Sedimentation and Counting Technique (SCT) are laborious, time consuming and can only be applied post mortem in the diagnosis of *Echinococcus multilocularis* infection in the final hosts. A magnetic capture real-time PCR (MC-qPCR) method has been proposed as an alternative for IST or SCT.
Objective: The main objective of the present study was to characterize the diagnostic performance of the MC-qPCR. In addition, we aimed at comparing results obtained by MC-qPCR, with those of a real-time PCR (qPCR) and a conventional gel PCR (cPCR).
Material & Methods: For the characterization of the MC-qPCR, samples collected from the *Ampulla recti* of 120 foxes were used. Based on the IST results, the samples were assigned to five groups: *E. multilocularis*-negative animals (n = 30), samples from animals infected with at least 1-5 adult *E. multilocularis* worms (n = 30), with 6-50 worms (n = 30); with 51-1000 worms (n = 20) and with more than 1000 worms (n = 10). DNA samples from these faecal samples were also extracted using commercial DNA extraction kits and amplified by qPCR and cPCR.
Results: In the experiments, the MC-qPCR showed the highest diagnostic sensitivity (91%; 95%CI: 83-96%) followed by qPCR (81%; 95%CI: 71-89%) relative to IST. The lowest diagnostic sensitivity was found for the cPCR (52%; 95%CI: 41-63%).
The diagnostic specificity of the qPCR and the cPCR relative to IST was 100% (95%CI: 88-100%). MC-qPCR revealed a diagnostic specificity of 93% (95%CI: 78-99%).
Conclusion: The MC-qPCR showed a good diagnostic sensitivity relative to IST, qPCR and cPCR, indicating that MC-qPCR may represent an promising alternative to IST.

EPI-P-04

Free-Living Amoebae as hosts for "Giruses" and vectors of microorganisms with "Public Health" significance

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Free living amoebae (FLA) pose a considerable risk regarding environmental health and public health significance. While FLA are known as parasites of both humans and animals causing a wide range of symptoms they can also act as vectors of phylogenetically diverse microorganisms, called

endocytobionts. Among those digestion- and lysis- resistant intracellular microorganisms there are human pathogenic microbes evoking individual medical problems and on a broader scope Public Health concerns. FLA often serve as vectors of those microorganisms from the environment to humans. The endocytobionts are transported and protected by the FLA, among them bacteria, viruses, protozoa or fungi. A considerable diversity within FLA was published recently representing a range of pathogenic water borne, food borne and soil organisms. The relationship between FLA and their endocytobionts has an influence on evolutionary processes, a fact, that could be demonstrated when detecting the so called "Giant viruses" (= giruses). Mimiviruses and Pandoraviruses are examples for interesting viral endocytobionts within FLA. The development of (human) pathogenicity and virulence is associated with FLA and intracellularly residing bacteria. Environmental and climatic changes (whether arising from nature or human influence) are affecting FLA abundance, which may lead to an increase of infectious diseases associated with FLA or their endocytobionts.

EPI-P-05

Molecular Epidemiology and Future Projection of Cutaneous Leishmaniasis in Libya Untill 2060

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Question: Cutaneous leishmaniasis (CL) is a major public health problem in Libya. In this paper, we describe the eco-epidemiological parameters of CL during January 2011 till December 2012. Current spatiotemporal distributions of CL cases were explored and projected to the future using a correlative modelling approach. In addition the present results were compared with our previous data obtained for the time period 1995-2008.
Methods and Results: We investigated 312 CL patients who came from 81 endemic areas distributed in 10 districts. The patients presented with typical localized CL lesions. Molecular identification of parasites by a PCR-RFLP approach identified two causative species: *L. major* and *L. tropica* comprised (72.8 %) and (27.2 %) of the cases, respectively. *Leishmania tropica* was found mainly in the three districts Murqub (27.3 %), Jabal al Gharbi (27.3 %) and Misrata (13.7 %), and *L. major* in Jabal al Gharbi (61 %) and Jafara (20.3 %). Seasonal occurrence of CL cases showed that most cases (74.2 %) occurred between November and March, *L. major* cases from November till January (69.4 %), and *L. tropica* cases mainly in January and February (41 %). Spatiotemporal projections using correlative distribution models based on current case data and climatic conditions showed that coastal regions have a higher level of risk due to more favourable conditions for the transmitting vectors.
Conclusion: Future projection of CL until 2060 showed a trend of increasing incidence of CL in the north-western part of Libya, a spread along the coastal region and a possible emergence of new endemics in the north-eastern districts of Libya. These results should be considered for control programs to prevent the emergence of new endemic areas taking also into consideration changes in socio-economic factors such as migration, conflicts, urbanization, land use and access to health care.

EPI-P-06

Cryptosporidium parvum and other intestinal parasites among diarrheal and non-diarrheal HIV positive patients in Asella teaching hospital, Ethiopia

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Introduction: In Ethiopia the prevalence of opportunistic parasites among HIV positive patients is under estimated as the routine wet mount microscopy of stool samples normally performed in health facilities is not sensitive enough to detect those parasites.
Objective: The main objective of this study was to determine the prevalence of opportunistic parasites and other intestinal parasites among HIV positive patients in Asella teaching hospital, Ethiopia.
Materials & Methods: An institutional based cross-sectional study design was implemented. Stool and blood samples were collected from 163 HIV positive patients. Stool examination for opportunistic parasite infections was done by wet mount and modified acid fast staining (AFS) after processing the stool samples with Telemann concentration technique. CD4 count was done by BD FACSCount™ Flow Cytometer. Socio-demographic data was collected using pretested questionnaire. Data was entered and analyzed by SPSS version 20.
Result: Majority of the participants, 101 (62.0%), were female and 62(38%) were males. The mean age of participants was 38.2(SD±10.7) years. The prevalence of intestinal parasitic infection was 30(18.4%). Protozoa (*E. histolytica*, *G. lamblia* and *T. homins*), helminths (*Taenia* species, *A. lumbricoides*, *S. stercoralis* and *H. nana*), and opportunistic intestinal parasites (*C. parvum*) were observed in 9 (5.5%), 8(4.9%), and 12(7.4%) patients, respectively. The prevalence of *C. parvum* was significantly higher among those patients with diarrhea (12.9%, p=0.005) and CD4 count lower than 200 cells/mm3 (25.9%, p=0.001). Having repeated contact to animal's excreta (AOR 2.53; 95% CI 1.01, 6.38) and having habit of eating uncooked food (AOR 5.40; 95% CI 2.18, 13.37) were significantly associated with intestinal parasitic infection.
Conclusion: No oocysts of opportunistic parasite were detected by the routine wet mount stool examination. Therefore it is mandatory to include the AFS technique into the regular laboratory service to enhance the quality of care for HIV patients in the hospital.
Key words: Opportunistic parasites, HIV, Ethiopia

EPI-P-07

Epidemiology of Crimean-Congo Hemorrhagic Fever in Livestock animals of Balochistan, Pakistan: A risk indicator for the human population

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Background: Crimean-Congo hemorrhagic fever (CCHF) is a severe, often lethal viral disease caused by the arbovirus Crimean-Congo hemorrhagic fever virus (CCHFV). It is mainly transmitted to humans and animals by Ixodid ticks of genus *Hyalomma.* Livestock animals serve as a reservoir for the virus, but do not show clinical signs. In Pakistan, about 35 million people are engaged in livestock related activities. In the province of Balochistan, more than 47% of the provincial economy is dependent on animal husbandry. Therefore more human CCHF cases are reported in this province than in other parts of the country. A common method for risk estimation for the human population is the analysis of the CCHFV prevalence in livestock. Currently, no such data is available regarding CCHFV or CCHFV specific antibodies in livestock in Pakistan.
Methods: A cross-sectional study was conducted to study the epidemiology of CCHFV in livestock animals of Balochistan, Pakistan. Blood samples were collected from 1600 sheep and goats in Quetta, Sibi and Zhob divisions of Balochistan from July to September 2016. Farm and animal related information was collected in moderated interviews using a standard questionnaire. Pools of serum samples were tested for CCHFV genome fragments by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). For serological analysis, two indirect CCHFV-IgG-Enzyme-Linked Immunosorbent Assays (ELISA) were used. In case of divergent results an adapted commercial immunofluorescence assay (IFA) was also performed.
Results: RT-q PCR identified 8 CCHFV positive serum pools (2.5%), while serological analysis revealed 5-8% goat samples as positive for CCHFV-specific IgG antibodies.
Conclusions: Goats form a reservoir for CCHFV in the study area. Risk factors for infection will be identified using the epidemiological data collected on the farms.
Keywords: Epidemiology, Crimean-Congo hemorrhagic fever, cross-sectional study, livestock, RT-PCR, serology, Pakistan

EPI-P-08

Parasitological investigation of *Eimeria spp.* and haemosporidia among domestic chickens (*Gallus gallus domesticus*) and guinea fowls (*Numida meleagris*) slaughtered at two selected poultry markets in Lagos State, Nigeria.

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Economic losses due to parasitic diseases are a major challenge posed against sustainable poultry production worldwide. This study determined the occurrence and prevalence of *Eimeria spp.* and

haemosporidia among domestic chickens and guinea fowls slaughtered at Oyingbo and Onipanu live-bird markets in Lagos State, Nigeria. Blood samples and intestinal contents at three distinct segments of the gut were collected from 30 domestic chickens and guinea fowls each at both markets. Wet smears of intestinal contents were microscopically examined for oocysts while thin films of blood were Giemsa-stained for the demonstration of protozoa. Histological changes were studied for the intestine sacrificed birds Results revealed that 19 (31.7%) and 21 (35%) chickens and guinea fowls respectively were positive for *Eimeria spp.* infection. There was no significant difference (P>0.05) in *Eimeria spp.* infections between the chicken breeds and also the sexes of both poultry. Oocysts of *Eimeria* were mostly recovered from the caeca and small intestines of guinea fowls and chickens respectively. A total of 20 (33.3%) chickens and 11 (18.3%) guinea fowls were infected with different blood protozoa namely, *Plasmodium spp.*, *Leucocytozoon spp.* and *Haemoproteus spp*. Higher prevalence rate of avian malaria infection was recorded among chickens (23.3%) than guinea fowls (15%). None of the sampled guinea fowls were positive for *Leucocytozoon spp.* Prevalence rates of the haemosporidian infections between breeds of chickens and the sexes of both birds were also statistically similar (P>0.05). Histhopathological assessments of affected caecal segment revealed the desquamation and atrophy of intestinal epithelial cells, necrosis of the glandular epithelium, and the presence of few unsporulated oocysts. Haemosporidia and *Eimeria spp.* are prevalent in both poultry markets considered in this study. Strict measures should therefore be taken to control these parasites, in order to prevent severe economic losses.

EPI-P-09

Comparative study of toxoplasmosis amongst healthy volunteers and Schizophrenics attending two Health Facilities in Port Harcourt, Rivers State, Nigeria.

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Abstract
Toxoplasmosis is a neglected tropical zoonotic infection caused by an intercellular protozoan parasite called Toxoplasma gondii. Toxoplasma gondii infection is gaining prominence as an important public health parasitic infection and possibly the aetiology of some cases of schizophrenia. The comparative seroprevalence and associated risk factors of toxoplasmosis were investigated among two sub populations - schizophrenics (SZN) drawn from the Neuropsychiatric hospital Port Harcourt and Neuropsychiatric department of University of Port-Harcourt Teaching Hospital, all in Rivers State. Immunocompetent persons (IP) were used as control after ethical clearance was obtained from Rivers State Hospitals Management Board Ethical Committee. Immunodiagnostic techniques involving the detection of T. gondii antibodies in examined sera using ELISA IgG and IgM tests were employed. Well structured questionnaire was used to collect data on social demographic risk factors associated with toxoplasmosis. Out of the 400 subjects (200 subjects per sub population) examined, SZN recorded a seroprevalence of 54.0% (109) ($P \leq 0.05$), while IP recorded 28.5% (57) ($P \leq 0.05$). SZN recorded a higher seroprevalence compared to IP with 50.0% (100), 4% (8) and 21.5% (43), 7.0% (14) for Toxoplasma gondii ELISA IgG and IgM tests respectively. Age groups 35-40 and 40 and above both had the highest seroprevalence 11.0% (22) for SZN while age groups 25-29 had the highest seroprevalence of 7.5% (15) for IP. Students recorded the highest seroprevalence for SZN, 21.5% (43) for IgGE and

2.0% (4) for IgME while traders recorded the highest seroprevalence for IP 6.5% (13) for IgGE and 2.5% (5) for IgME. More males were infected among SZN while more females were infected for IP. Eating improperly washed fruits and vegetables, and drinking untreated water were the risk factors associated with the disease. Public health campaign, improved personal hygiene and routine tests have been advocated for.

Keywords: Rivers State, seroprevalence, schizophrenia, toxoplasmosis, Nigeria.

Figure 1

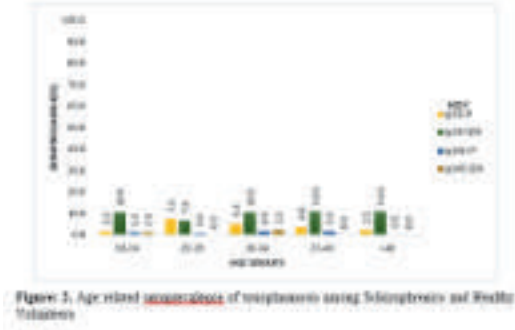
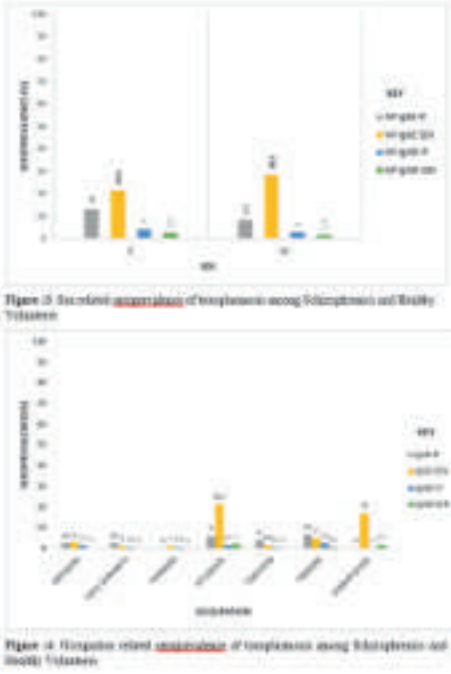


Figure 2



EPI-P-10

Retrospective analysis of vector-borne diseases in dogs after travelling to endemic areas (2007-2015)

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Introduction: Canine vector-borne diseases gained in importance in Germany due to climatic changes, growing tourist traffic and the increased import of dogs from abroad. Endemic regions for pathogens such as *Leishmania* (*L.*), *Hepatozoon* (*H.*), *Ehrlichia* (*E.*) and *Dirofilaria* (*D.*) are the Mediterranean area and South Eastern Europe. *Anaplasma* (*A.*) and *Babesia* (*B.*) species are present all over Europe.
Objectives: The objective of this retrospective study was to evaluate whether dogs were exposed to a corresponding risk of infection when travelling to endemic regions.
Materials and Methods: The study included the results of the primary consultation of 227 patients attending the Small Animal Clinic FU Berlin between 01/2007 and 12/2015, who had accompanied

their owners to 17 endemic countries (13 Mediterranean countries, 4 countries in South Eastern Europe[rme1]). A total of 1113 tests were sent to external laboratories (579 direct / 534 indirect methods of detection).
Results: The positive results were: *A. phagocytophilum* 15/158 dogs (9.5%; PCR 5/149; IFAT 11/38), *E. canis* 13/169 (7.7%; PCR 2/45; IFAT 13/157), *L. infantum* 9/195 (4.6%; PCR 4/42; IFAT 7/181; ELISA 0/12), *B. canis* 8/176 (4.5%; PCR 2/93; IFAT 4/118; ELISA 2/17), *B. gibsoni* 2/99 (2%; PCR 2/90; IFAT 0/11). None of the dogs was tested positive for *D. immitis* (ELISA 0/79), microfilaria (PCR 0/6), *H. canis* (PCR 0/21) or *A. platys* (PCR 0/5). The Knott-test was positive in 0/47 dogs.
Conclusion: 39/227 (17,2%) of the tested dogs were positive for at least one pathogen. Prevention of vector transferred pathogens in dogs travelling to national and international endemic regions is of great importance.

EPI-P-11
Characterization of *Trypanosoma* sp. circulating in Nigeria and Southern Chad

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Trypanosomes are protozoan parasites causing Trypanosomiasis in both humans and livestock worldwide. African trypanosomes are the cause of sleeping sickness in humans and Nagana in cattle in sub-Saharan Africa. The diseases are most prevalent in rural and poorly developed regions, where Nagana is responsible for a disastrous loss of livestock animals.
Humans and animals get infected by the bite of an infected tsetse fly, the sole vectors of African trypanosomes. To complete their life cycle, trypanosomes rely on the cyclic transmission between the tsetse fly vector and the mammalian host.
Due to restricted access to remote areas, not much information is available about the distribution of trypanosomes in many African countries. The diversity of *Trypanosoma* species present is poorly documented, and often relies on microscopical analysis, a technique prone to misidentification.
Surveys were conducted in Nigeria and in HAT foci of Southern Chad. During a country wide survey in Nigeria, tsetse flies were analysed for presence of trypanosomes. In an epidemiological survey in Chad, blood samples were collected from humans in two foci to analyse exposure to trypanosomes. A second survey is focussing on distribution of trypanosomes in tsetse flies and cattle. Molecular analysis was used to investigate the diversity of *Trypanosoma* species by Internal Transcribed Spacer-1 [1,2]. Phylogenetic analysis of trypanosomes by glycosomal GAPDH was performed to investigate the segregation of populations in different locations [3].
In Nigeria, a high diversity of different *Trypanosoma* species was found in tsetse flies. Noticeable is the predominant presence of *T. grayi*, a reptile trypanosome. In Chad, positive cases were found in human samples.
The results can be used as a base to develop strategies towards the eradication of Trypanosomiasis. The high diversity indicates that continuous surveys and the combination of molecular tools with parasitological methods are necessary to identify the *Trypanosoma* species present and correctly treat the diseases.

[1] Adams et al. 2006, doi10.1016/j.actatropica.2006.10.002
[2] Ngomtcho et al. 2017, Parasit Vectors (in print)
[3] Hamilton et al. 2004, doi10.1016/j.ijpara.2004.08.011

EPI-P-12

Screening of Mediterranean tick species by high-throughput microfluidic real time PCRs reveals a significant diversity of bacterial and parasitic pathogens with co-infections being the norm

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Question: A number of recent studies has increased awareness of co-infections on both the tick and host level. This emphasizes the need for the utilization of diagnostic tools, which efficiently allow simultaneous monitoring of human and/or animal pathogens. Aim of the present survey was to screen a significant number of individual Mediterranean tick species collected from farm animals in Greece for the presence of 39 pathogens/symbionts by employing a high-throughput microfluidic real-time PCR method (BioMark™ real-time PCR system/Fluidigm, USA).
Methods: 1101 adults ticks and 75 nymphs belonging to the genera *Rhipicephalus*, *Haemaphysalis*, *Hyalomma*, *Dermacentor* and *Ixodes* (11 species) were analysed by the above method. They were collected from sheep, goat and cattle farms (n=113) located on the island of Lesvos (east), Crete (south) and along the northern border of Greece. Ticks were screened for the presence of 27 bacteria and 12 parasites belonging to the genera *Borrelia, Anaplasma, Ehrlichia, Rickettsia, Bartonella, Candidatus Neoehrlichia, Coxiella, Francisella, Babesia, and Theileria.*
Results: 75.4% of the ticks were infected with at least one pathogen. Mixed infections seem to be very common, as 50.5% of the ticks were harbouring more than one pathogen and/or symbiont, whereas in some cases (1.4% of ticks) infections with up to 5 pathogens and/or symbionts were observed. All in all 14 bacteria (*Rickettsia aeschlimanni*, *R. massiliae, R. slovaca, R. conorii, Anaplasma ovis*, *A. phagocytophilum*, *A. marginale*, *A. platys*, *A. centrale*, *A. bovis, Ehrichia canis, Bartonella henselae*, *Coxiella burnetii* and *Franciscella tularensis* with the latter 2 being either pathogens or symbionts) and 5 parasites (*Babesia vogeli*, *B. caballi*, *B. major*, *B. bigemina* and *B.ovis*) were identified during this study.
Conclusions: Our results suggest that co-infection in Mediterranean tick species is the rule rather than the exception, thus raising questions about possible co-transmission of these agents to humans and/or animals. This highlights the necessity to better understand underlying interactions of both pathogens and symbionts with the ultimate goal of considering them in the development of new control strategies against ticks and tick-borne diseases.

EPI-P-13

Apicomplexan and helminth parasite infections of free-ranging cheetahs (*Acinonyx jubatus*) on Namibian farmland

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Introduction: Cheetahs display a low genetic variability, including at the major histocompatibility complex (MHC) which is an important gene group linked to the immune system. Thus, cheetahs would be expected to be susceptible to infectious diseases and parasites. However, free-ranging cheetahs have low disease prevalence indicating an effective immune system. Although several studies reported exposure to different viruses and bacteria in different free-living cheetah populations, information on parasites is lacking.
Objectives and Methods: Here we assess the potential disease risks of parasites of the largest free-ranging cheetah population in the world, which lives on farmland in Namibia. We screened sera of 255 free-ranging cheetahs for antibodies against the Apicomplexan parasite species *Toxoplasma gondii*, *Neospora caninum* and *Besnoitia besnoiti,* and investigated 39 faecal samples for gastrointestinal parasite stages.
Results: We detected in 49.4% of the samples antibodies against *Toxoplasma gondii*, whereas no antibodies were detected against the other two Apicomplexan parasites. Adult cheetahs had a significantly higher seroprevalence than juveniles. Coproscopic examinations detected oocysts of *Isospora* spp. and *Toxoplasma gondii* and eggs of the six helminth species *Ancylostoma* spp., *Toxascaris* spp., *Physaloptera* spp., *Taenia* spp., *Spirometra* spp. and *Dipylidium* spp., with total egg numbers ranging from 0 to 12350 eggs per gram of faeces. Neither sex nor age significantly affected number of *Ancylostoma* eggs, the most prevalent parasite. None of the cheetahs displayed clinical symptoms at the time of capture.
Conclusion: Our results suggest that the immune system of the cheetahs mounted an adequate response to the detected parasites because all infections were asymptomatic. Thus, cheetahs do not seem to be hampered by their low MHC variability. This is encouraging for the conservation of this largest free-ranging population.

EPI-P-14

***Baylisascaris procyonis* in free-ranging raccoons (*Procyon lotor*) in Saxony, eastern Germany**

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Introduction: The raccoon is an introduced species in Germany. In their native region, raccoons carry the zoonotic nematode *Baylisascaris procyonis*. *B. procyonis* can produce severe to fatal parasitosis in the brain of paratenic hosts: small mammals and birds. Humans can be infected by accidental oral

infection of embryonated eggs. In Germany, *B. procyonis* prevalence varies throughout the country. In central Hessen, there is a reported 71% of *B. procyonis* prevalence in free-ranging raccoons. And a single study claims *B. procyonis* to be absent from the north-eastern states of Brandenburg and Mecklenburg Western Pomerania. The objective of this ongoing study is to investigate the prevalence of *B. procyonis* in free-ranging raccoons from the eastern state of Saxony. This abstract presents some of the preliminary results.
Methods and preliminary results: From autumn 2017 to spring 2018, hunted raccoon carcases are being collected from the outskirts of Leipzig metropolitan area, northwestern Saxony, as well as surrounding regions. The entire gastro-intestinal track, including any faecal content, is removed. Intestines are complete dissected, large nematodes are collected, and morphological identification of *B. procyonis* is conducted. So far, 27 raccoon carcasses have been examined and *B. procyonis* has been found in 22 animals.
Conclusions and prospective outcome: We report the presence of *B. procyonis* in raccoons from the Leipzig area. The results described in this study are part of an ongoing project. The number of raccoon samples will increase in the following months and molecular analysis of *B. procyonis* worms will be performed. Since raccoons are commonly attracted to anthropogenic settlements, the risk of human infection is considerable; particularly for wildlife professionals, veterinarians, and hunters.

EPI-P-15
Prevalence of *kdr*-genotype of German headlice and relevance for pediculosis treatment

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Introduction: Infestations with *Pediculus humanus* var. *capitis* are a relevant Public Health issue. Treatment efficacy seems decreasing and emergence of resistant lice in particular to pyrethroids is a suspected reason. Non-synonymous mutations in the gene encoding the α-subunit of a voltage gated sodium channel (VSSC), so-called knock-down resistance (*kdr*) mutations, are implicated in pyrethroid resistance in insects. The *kdr*-genotype was previously described to occur in ~9 of 10 headlice from Germany but was found not to correlate with failure of a pyrethroid-containing pediculocide in a sponsored-study setting.
Objectives: Determine current *kdr*-mutation frequency and population structure of headlice in Germany and relate this to treatment outcome of pediculocides used by patients.
Material & Methods: Questionnaires on patient demographics, on chosen therapy and success as well as louse sampling sets were distributed via local health authorities. Study participants were invited to send back questionnaires and headlice or nits. Samples and questionnaires obtained were evaluated using STATA software and DNA amplicon analyses, respectively. *Kdr* and PM2 and S2 intergenic region genotyping was performed using published protocols and sequences analysed using Geneious software.
Results: Headlice were obtained from 206 patients and, where possible, 2 individual headlice/egg-containing or empty nits per patient were processed. In total 167 headlice could be genotyped. All except one were homozygous for *kdr*. For PM2 sequences, 10 genotypes were observed with 119 of 130 samples belonging to genotype EU928850. Only two S2 genotypes were observed with one being represented by a single sample. Questionnaire data of 135 cases indicated that pyrethroid-based

pediculocides are used in roughly 4 of 10 cases with treatment success comparable to that of non-pyrethroid-based, UBA-listed pediculocides.
Conclusion: Prevalence of the *kdr*-genotype of headlice currently approaches 100%. The mutations are found in genetically distinct fractions of the parasite population. Observational findings agree with previous data that the *kdr*-mutation is unlikely a major cause of treatment failure.

Free topics

FTO-P-01
Parasitological field work in an era of hyper regulation and the role of Museums collections

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In the "good old days", field work for parasitologists used to depend mostly on the explorer"s financial ressources and…. survival skills! Today"s exploration of parasitological biodiversity has become much more complex from a logistical point of view because the approval of numerous regulatory bodies is usually required at each stage of such project.
From the establishment of formal cooperation agreements to permits to access protected areas, from authorization to collect vertebrate hosts to rules for transporting their parasites in planes, not to mention respecting international regulations like the Nagoya protocol, each step of such scientific expedition has become a bureaucratic nightmare.
Obviously these regulations are mostly well-intentioned and can often be defended when taken individually. Still, their cumulative effects, added to their often wide and inappropriate scope make them a powerful brake to biodiversity discovery. In the case of parasites, dealing with hosts and parasites, which are two categories of organisms that are often treated differently, only makes the matter worse.
In this context, one might imagine that exploring previously collected material, especially those conserved in Natural History Museum collections, might be a good substitute for fresh sampling.
Using a practical example from Australia we show that, although some exploitable information can be derived from such collections, this is clearly not the case because their diversity and quality only make them a poor alternative to actual fieldwork. In our case, a survey of bird tapeworm collections in that country allowed the description of several new taxa, but also painfully demonstrated how poorly the diversity of these parasites is known and how useful and urgent it would be to facilitate new collecting.

FTO-P-02
Effect of different host plants utilization on physicomorphic responses of polyphagous *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera)

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The effect of host plants on herbivore insects is of great importance for their fitness. *Helicoverpa armigera* (Noctuidae; Lepidoptera) is a serious and widely spread insect pest of various crops being

polyphagous in nature, having higher fecundity and migrating efficiency. We conducted this study during 2016 at University of Sargodha (Pakistan) to check physicomorphic performance of *H. armigera* towards different host plants including (*Abelmoschus esculentus* (L.) (okra), *Cicer arietinum* (L.) (gram), *Solanum lycopersicum* (L.) (tomato) and an artificial diet. *H. armigera* got the highest larval survivorship (97.23%), larval and pupal weights (0.38g and 0.22g) during feeding on gram. The highest consumption index (CI) (1.49 %) was also observed in gram feeding and the lowest one was noted in tomato (0.95 %) feeding. Moreover, relative growth rate (RGR) and relative consumption rate (RCR) were found maximum (2.09mg/mg/day and 10.14 mg/mg/day) in case of gram herbivory. Similarly, the efficiency of conversion of ingested food (ECI) was also found the highest (70.65%) in gram feeding as compared to other host plants. In case of insect morphology parameters, the fore and hind wing areas (0.98 cm2 and 0.79 cm2) and the length of hind tibia (0.82 cm) were significantly greater in the adults of gram feeders. It is concluded that *C. arietinum* (gram) was proved a highly nutritious food plant among natural host plants, promoting *H. armigera* for its development and survival.

Immunology

IMM-P-01

Effect of the antimalarial drug pyrimethamine on the resolution of experimental cerebral malaria.

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Introduction: Cerebral Malaria (CM) is a severe complication of human malaria. Experimental cerebral malaria (ECM), caused by *Plasmodium berghei* ANKA (*Pb*A), is the widely used rodent disease model to study the pathogenesis of human cerebral malaria. The drug, pyrimethamine, has been used successfully to treat malaria. However, the effect of the drug on the resolution of cerebral malaria associated brain pathology remains elusive.
Objective: To visualize and characterize the effect of the drug pyrimethamine on the resolution of ECM.
Materials and methods: C57BL/6 mice were injected with *Pb*A-infected red blood cells. One group of mice was treated with pyrimethamine starting at day 5 post infection (p.i.) while the control group received phosphate buffer saline (PBS). Parasite load in the peripheral blood was enumerated daily until day 14 p.i. Intracerebral accumulation of CD8+ T cells was analysed by flow cytometry. Blood brain barrier (BBB) integrity was assessed by Evans blue staining. Cerebral chemokine and cytokine expression was measured by qRT-PCR. Brain pathology was studied by immunohistochemistry. Single-photon emission computed tomography (SPECT) was performed to visualize the changes in regional cerebral blood flow.
Results: All of the pyrimethamine treated mice were protected from ECM while the control mice succumbed to the infection. Pyrimethamine treatment reduced the parasite burden in the erythrocytes, impaired chemokine and cytokine production resulting in reduced cerebral accumulation of CD8+ T cells. Evans blue injection showed minimal damage to the BBB. Pyrimethamine ameliorated brain pathology with reduced neuroinflammation and haemorrhage as well as reduced endothelial cell

activation and apoptosis. SPECT imaging of control mice showed heterogeneous and diffused hypo-perfusion in the olfactory bulbs and cortical region, while pyrimethamine treated mice showed normal cortical perfusion in the olfactory bulbs and cortex.
Conclusion: Our study delineates the pathophysiological alterations taking place in the brain upon pyrimethamine treatment, thereby providing a better understanding of the disease resolution. These data could be useful for the development of adjuvant therapies for CM.

IMM-P-02
Preclinical evaluation of transgenic *Plasmodium berghei* sporozoites expressing the TLR5 agonist *Salmonella enterica* flagellin

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Lasting vaccine-induced immunity against *Plasmodium* parasites is difficult to achieve. Vaccination with metabolically active, attenuated sporozoites mediates sterile protection by activation of CD8+ T cells and the release of perforin and INFγ. Here, we evaluate the adjuvant potential of the TLR5 agonist flagellin (FliC) from *Salmonella enterica* to improve immunogenicity of *Plasmodium* sporozoite vaccinations. We designed recombinant *Plasmodium berghei* parasite lines that express flagellin under the control of the *UIS4* promoter in sporozoites and hepatic stages. Amino-terminal addition of a signal peptide permits secretion of flagellin in sporozoites and targeting to the parasitophorous vacuole in liver stages, thereby potentially eliciting a TLR5 dependent innate immune response. These transgenic parasite lines were tested in sub-protective vaccine protocols for their potential to induce superior protection as compared to normal sporozoites.

IMM-P-03
The importance of *Mansonella perstans* infection – an immuno-epidemiological study in Ghana

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Mansonella perstans is a human filarial parasite endemic in Sub-Saharan Africa and it is estimated that more than 100 million individuals are infected. The clinical manifestation of Mansonelliasis is not very distinct and severe and in most affected countries, including Ghana, largely neglected and not considered as public health problem. Therefore, up to now, epidemiological and immunological studies on *M. perstans* infections are scarce. Additionally standard drugs used to treat other filarial

parasites have shown limited efficacy against *M. perstans*, but a new treatment strategy using doxycycline targeting *Wolbachia* endosymbionts has been proposed.
Helminth parasites are known to induce immune-modulation and to polarize the immune response towards a type-2 response, which also affects immunity to other pathogens such as mycobacteria. Based on the fact that *M. perstans* infection causes only subtle clinical symptoms, we hypothesised that this helminth parasite evolved very efficient mechanisms to modulate the host immune system to allow survival. We performed an immmuno-epidemiological study *i)* to determine the actual prevalence of *M. perstans* in Ghana and the population at highest risk of infection; *ii)* to investigate the potential of doxycycline treatment to reduce *M. perstans* microfilariae and *iii)* to investigate immune-modulation induced by *M. perstans* and the consequences of doxycycline treatment on it and *iv)* to investigate the influence on mycobacterial co-infections.
In cross-sectional approach we first screened about 2200 people for the prevalence of *M. perstans* microfilariae in three districts in the middle belt of Ghana. Subsequently an interventional study was performed to determine the efficacy of doxycycline treatment on *M. perstans* infection and followed over two years. At baseline, 6, 12 & 24-month post-treatment immunological parameters were determined to investigate the influence of *M. perstans*.
Data presented will show that *M. perstans* is highly prevalent in Ghana and doxycycline efficiently reduces microfilariae. Finally we will present data on immune modulation and the how this affected by treatment. In conclusion, this study provides more insights into this filarial infection, which so far has been largely neglected

Funded by the DFG.

IMM-P-04
Immune reactions against the laboratory strain of Eimeria falcifomis are stronger than against a wild derived strain

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Infections with different species of the genus Eimeria (Apicomplexa: Coccidia) can have different dynamics, vary in the immune reactions they induce and in pathology they imposed on their hosts. Here, we investigate dynamics of oocyst shedding, cytokine profiles, tissue inflammation and cellular immune response in infections of mice with wild derived strains of E. flaciformis (falW) and E. ferrisi (ferW) and E. flaciformis BayerHaberkorn (falL), a strain propagated in the laboratory for over 50 years through experimental passaging. The two different species E. flaciformis and E. ferrisi have different infection daynamics in NMRI mice: after a pre-patency period of only 3 days oocyct shedding of ferW peaks in intensity at 6 days post infection (dpi) and continues only to 7 dpi. In contrast, after pre-patency of 5 day, oocyst shedding of falL and falW has a later peak intensity at 8 dpi and 9 dpi, respectively. Gene expression of the cytokines CXCL9, IL6, IL10 and IL 12, TGF-β and the transcription activator STAT6 is significantly increased in infections with falL and all those genes are detected at significantly lower levels in infections with the two wild derived strains falW and ferW. Inflammation manifested in a mixture of granulocytes and mononuclear cells in the ceca and the area occupied by leukocyte infiltration was larger in infections with falL than in those with both wild derived strains

(falW and ferW). We can conclude that NMRI mice mount a stronger immune and inflammatory response against the laboratory strain of E. flaciformis compared to both wild derived lines of E. falciformis an E. ferrisi. It seems possible that evolution during laboratory passages produced a parasite strain, which still resembles a closely related wild derived strain in infection dynamics, but drastically differs in the immune response it induces. Immune response against laboratory and wild derived strains of E. falciformis differs to an extend, that the latter is more similar to the response against a different species of the parasite. These result show that caution is needed when using laboratory strains of pathogens to draw conclusions about infections in natural systems.

IMM-P-05

Host age and background affect the phenotype of GATA-3 expressing CD4+ T cell in nematode infection

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Introduction: Helminths infect around 30% of the human population worldwide. The dominant immune response to helminths is characterized by type 2 T helper (Th2) essential for protection. We have previously shown that many helminth-reactive CD4+ T cells display a Th2/1 phenotype by co-expressing the lineage-specifying transcription factors GATA-3 and T-bet as well as Th2 cytokines and IFN-γ.
Objectives: We assessed if the proportions of Th2/1 and conventional Th2 cells generated in infections with the enteric nematode *Heligmosomoides polygyrus* differ depending on the route of infection, gut microbiota or larval burden, and host sex, age or background.
Material & Methods: T-and B-cell responses of infected mice were surveyed by flow cytometry, qPCR and serum antibody profiles.
Results: To survey if the oral route of infection promoted the instruction of Th2/1 cells, T cell responses of mice orally infected with stage 3 larvae were compared to those of mice immunized subcutaneously with either L3 or excretory/secretory worm products. Th2/1 differentiation was similar in all conditions. Furthermore, infected germ-free mice generated normal Th2 and Th2/1 hybrid responses, arguing against a dependency of Th2/1 instruction on microbial signals. Th2/1 differentiation robustly occurred in response to low and high worm burdens. While both sexes generated similar ratios of Th2/1 hybrids and Th2 cells, Th2/1 differentiation was reduced in more Th2-prone BALB/c mice compared to C57BL/6 or Sv129 mice. Our preliminary data suggest that host age also affects the T effector response. Compared to young mice, aged individuals tended to develop poor conventional Th2 responses, while Th2/1 differentiation was unimpaired.
Conclusion: Th2/1 hybrid cells are a prominent population within the helminth-reactive CD4+ T effector pool. Whether proportions of Th2/1 hybrid cells account for different effectiveness of immune responses to nematode infections is the focus of ongoing work.

IMM-P-06

***Cryptosporidium* Infection and Correlation with CD_4+ T-cell count among HIV on HAART therapy in Osogbo, Nigeria**

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Cryptosporidiosis is the chief AIDS-defining infection in no more than 2% of HIV reported cases. This study was carried out to determine the prevalence of *Cryptosporidium* infection and its correlation with CD_4+ T-cell among HIV individuals in Osogbo. A total of 188 HIV seropositive patients attending IHVN Clinics, LAUTECH, Nigeria and 60 HIV negative individuals were selected for the study from January to December, 2014 by random sampling technique. Stool samples were collected and examined for *oocyst* and antigen of *Cryptosporidium protozoan* using Ziehl Neelsen (ZN) and ELISA methods respectively. Blood samples were analysed for CD_4 cell count by flow cytometry. Data were analysed using SPSS and P value < 0.05 was considered significant. Prevalence of 28.7% (54/188) and 35.1% (66/188) was obtained by ZN and ELISA respectively in HIV positive subjects while that of HIV negative was 1.7% (1/60). There was a significant association between: *Cryptosporidium* infections and diarrhoea (P= 0.004, χ^2=59.876, df=10), *Cryptosporidium* infection and age group (P =0.021, χ^2= 9.758, df = 4), *Cryptosporidium* infections and diarrhoea duration (P=0.001, χ2=105.223, df=1) and *Cryptosporidium* infections and CD4 cell counts (P = 0.001, χ^2=56.231, df=10,).*Cryptosporidium* infection was highly prevalent among HIV seropositive individuals in Osogbo, an indication of active infection that is likely to emerge as major human pathogen in a poor social economic setting. This study re-emphasized the need for inclusion of *Cryptosporidium* screening and treatment in HIV seropositive subjects since it is a major cause of morbidity and mortality.

Keywords: *Cryptosporidium spp,* CD_4 cell counts, ELISA, Microscopy, HIV/AIDS

IMM-P-07

Acute toxoplasmosis and the hyporesponsiveness of splenocytes

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Toxoplasma gondii (*T. gondii)* is an obligate intracellular parasite of medical and veterinary importance. Up on infection, *T. gondii* triggers a type 1 T helper cell immune response, whereby the major mediator of resistance to infection is interferon-gamma (IFN-γ). A broad spectrum of IFN-γ induced effector mechanisms including immunity related GTPases, guanylate binding proteins, inducible nitric oxide synthase and indoleamine 2,3-dioxygenase (IDO) contribute to the inhibition of replication and eradication of the intracellular pathogen and thus contribute to the control of an acute toxoplasmosis.
However, concomitant with an acute toxoplasmosis suppression of T-lymphocyte responses that diminish after several weeks is observed. Similar immunosuppressive effects are observed in other

infectious diseases, including viral, bacterial (e.g. tuberculosis) and other parasitic infections (e.g trypanosomiasis, leishmaniasis and neosporosis).
Characterization of the hyporesponsiveness during an acute toxoplasmosis has not been completely achieved. Nitrite oxide (NO) synthase activity has been shown to contribute to the suppression of the responsiveness during acute toxoplasmosis, nevertheless not being the sole cause. IDO activity, another IFN-γ induced effector mechanism, has also been described to possess immunoregulatory properties, but its role in the altered responsiveness to mitogens during acute toxoplasmosis is obscure. Thus, the defective response of splenocytes following intraperitoneal infection of C57BL/6 and IDO-deficient mice with *T. gondii* tachyzoites was analyzed. In this context, primed and unprimed splenocytes isolated from infected and naïve mice, respectively, were stimulated *ex vivo* with the mitogen concanavalin A. To further discriminate between cell-cell mediated interactions and soluble factors (e.g. cytokines like IL-2 and IL-10) that influence splenocyte proliferation cell-mixing experiments and supernatant transfer, respectively, were used. The proliferative capacity of splenocytes was assessed by measurement of incorporated radioactive thymidine.

Molecular genetics

MOL-P-01

RNAi mediated knockdown of targeted genes in *Strongyloides ratti*

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The model parasitic nematode *Strongyloides ratti* has been largely resistant to reverse genetic analysis. Gene knock down by RNA interference was unsuccessful and while CRISPR/Cas9 mutagenesis was recently reported for *S. stercoralis* and *S. ratti,* the low efficiency meant that no mutant strains of *S. ratti* were established. The lack of success can in part be attributed to a high mortality after microinjecting *S. ratti,* due to the high internal gonad pressure, and the high abortion rate of *S. ratti* embryos when kept on agar plates as is normal after microinjections. After optimizing the culture conditions thereby increasing embryonic and larval survival on plates, I established a protocol for RNAi by soaking instead of injection based on published protocols for *Brugia*.
Protocol in brief: L4 adults were isolated from fecal cultures by Baermann funnels, washed and placed singly into RPMI-1640 for 1 hour. Per sample 10 females and 10 males with high motility were picked into tubes containing 100µl RNAi-culture medium (siRNA 5µM, 10U RNaseOUT, RPMI-1640), placed into a 50ml tube with 4 others and surrounded with 35ml RPMI-1640 and incubated at 19°C. Two siRNAs targeting the gene *daf-12*, a scrambled control siRNA and the RNA dissolving buffer as a negative control were tested. Every 24 hours, tubes were removed, briefly centrifuged and then pipetted onto an optimized plate. Groups of individuals were isolated into Trizol for qPCR, with the remaining individuals and offspring screened for phenotypic changes.
daf-12 expression was reduced continually over the course of the experiment, with near total suppression (FC 0,01) achieved after 5 days of soaking. To test for off-target effects, 2 paralogs of *daf-12* as well as a gene with some similarity to the siRNA target sites were tested by qPCR with no significant changes in expression found. Whilst a visible phenotype was not detected, experiments are still ongoing to determine changes in fatty acid metabolism.

MOL-P-02
New molecular tools for studying the biology of *Leishmania mexicana*

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The genus *Leishmania* unites parasitic protists of the family Trypanosomatidae causing leishmaniases, closely related diseases that affect human and animal populations mainly in the tropical and subtropical regions. The clinical manifestations vary from spontaneously healing skin lesions to progressive and possibly fatal visceral infections. Leishmaniases represent a global health problem with over 350 million people at risk and an annual incidence rate of 2–10 million worldwide.
Several molecular tools have been developed in recent years to study *Leishmania mexicana*, a causative parasite of cutaneous leishmaniasis. These methods have greatly extended knowledge concerning functions of numerous genes and their association to *Leishmania* virulence. What more, some approaches provided an elegant way to control parasite´s gene expression at the level of RNA or protein stability. Here, we present three novel additions to the "*Leishmania* toolbox", namely an improved CRISPR/Cas9-, a Tetracycline-inducible gene expression- and inducible protein stabilization systems, all established in *Leishmania mexicana*. Advantages and limitations, along with potential applications will be also discussed.

MOL-P-03
Development of CRISPR/Cas9-based reverse genetics for *Leishmania braziliensis* and application to the study of differentially expressed target genes.

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Introduction: *Leishmania braziliensis*, the main cause of cutaneous and mucocutaneous leishmaniasis in South America, has so far eluded reverse genetic analyses. The presence of a functional RNA interference (RNAi) pathway in this species has opened the way for studying gene function in this important human pathogen. However, RNAi is prone to off-target effects. Recently, the CRISPR-Cas9 system has emerged as a highly efficient genetic engineering tool for genome editing in diverse eukaryotic cells, including Old World leishmaniae, but not New World *L*. (*Viannia*) species.
Objectives: We seek to establish the CRISPR-Cas9 technology in *L. braziliensis* and test its applicability by targeting endogenous genes encoding heat shock proteins HSP100 and HSP23, which were shown to be amenable for gene replacement in Old World *Leishmania* giving rise to conditional, viable phenotypes.
Methods: Strains of Peruvian *L. braziliensis* are being engineered to express Cas9 nuclease and T7 RNA polymerase using integration plasmids of the pIR series. Cell lines will then be co-transfected with donor DNA with 30 nucleotide homology flanks and two single guide RNA (sgRNA) templates for the corresponding target gene, *Hsp100* (*clpB*) or *Hsp23*. The genotypes and phenotypes of viable double

antibiotic-resistant transfectant cell lines will be characterized *in vitro* and compared with wild-type cells.
Results: Current work with the *L. braziliensis* strain PER005cl2 demonstrated that it can undergo homologous recombination effectively, as shown by the expression of green fluorescent protein from the integrated pIRmcs3+ vector that was originally developed for *L. major*. This already opens the way for targeted expression of Cas9 and T7 RNA polymerase in *L. braziliensis.*
Conclusion: Our results show that integration of transgenes into the *L. braziliensis* 18S rRNA locus is feasible and produces the intended gene product(s), allowing us to proceed.

MOL-P-04
MT10 is the most common Microsatellite Type (MT) among *Trichomonas vaginalis* isolates from Aydın, Turkey.

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Introduction: *Trichomonas vaginalis* (*T. vaginalis*), an anaerobic protozoan parasite of humans, is one of the most common sexually transmitted pathogen in the world. However, there is still insufficient information about the genetic properties of *T. vaginalis*.
Objectives: In this study, for the first time, genetic diversity of *T. vaginalis* isolates from Turkey was determined by using polymorphic microsatellite loci. Moreover, we also created a new database for microsatellite types (MT) for identification and allelic comparison of microsatellite types for the use of researchers from different countries.
Materials & Methods: A total of 30 *T. vaginalis* isolates from Aydın, Turkey were used in the present study. Following DNA isolation, the microsatellite locus of each isolate was amplified by polymerase chain reaction (PCR). The lengths of these loci were determined by fragment analysis. The loci used for MT typing were selected and allele numbers were given for each locus according to the length of the fragment. The MT numbers of the isolates were determined according to allelic profiles of loci.
Results: Analysis of the results showed presence of 16 microsatellite loci, 1 (6.3%) monomorphic and 15 (93.7%) polymorphic, that were amplified from *T. vaginalis* isolates. Of 15 loci 8 were used for microsatellite typing. It was found that the studied microsatellite loci were 2 to 6 nucleotides long and had 3 to 9 alleles. Among 30 isolates, 2 (6.7%) were MT3; 5 were (16.7%) were MT10 and 3 were identified as MT18 (10%). All remaining isolates (66.6%) were given different types of numbers because they differ from the others in at least one locus.
Conclusion: This is the first study on the identification of the genetic structure of *T. vaginalis* in Turkey and showed that these isolates vary in their microsatellite loci. In this study, typing of *T. vaginalis* was determined using microsatellite loci and MTs were presented, the most common MT was found to be MT10. Using our database global dissemination of *T. vaginalis* MT types can be determined.
This study was supported by TUBITAK 3001 with the project number 215S654.
Key words: *Trichomonas vaginalis*, microsatellite, genotype, microsatellite typing

MOL-P-05

Protein expression of *Toxoplasma gondii* recombinant protein (SAG1 and Cyc18) in *Leishmania tarentolae*

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Abstract

Leishmania tarentolae has been used successfully for a protein expression as it is simple and inexpensive to grow, but has the ability to glycosylate proteins. Herein we construct a novel filamentous expression system for use in *Leishmania tarentolae* with the aims of improving yield and facilitating purification. To achieve this, filamentous structures (consisting of secreted acid phosphatases SAP1 and SAP2) which are naturally expressed and secreted in abundance by *Leishmania mexicana* promastigotes were used as carriers of recombinant vaccine candidates for *Toxoplasma gondii*. These filamentous structures self assemble from the acid phosphatase subunits and are secreted from the flagellar pocket of *Leishmania*. For proof of principle SAG1 and Cyc18 proteins from T. gondii were chosen. SAG1 is the major surface protein of *T. gondii* and has been used with some degree of success in previous experimental vaccine and Cyc18 is known to be a CCR5 agonist which induces IL-12 production and has the potential to serve as natural adjuvant.

Methods:

The following steps were taken:

1. Generation of plasmid constructs for expression of filamentous secreted acid phosphatase fused with SAG1 (SAP1/SAG1) and Cyc18 (SAP1/Cyc18).
2. Protein expression determined by immunofluorescence and immunoblot analysis using the specific mAb LT8.2 against a peptide epitope and anti-His-tag antibodies.
3. Purification the filamentous proteins by using ammonium sulphate from the culture supernatant and detect the activity of phosphates (SAP) enzyme.

Results:

1- Two *L. tarentolae* cell lines were successfully generated carrying expression constructs for of filamentous secreted acid phosphatase fused with SAG1(SAP1/SAG1) and Cyc18 (SAP1/Cyc18).

2- Expression of SAP1SAG1protein in *Leishmania tarentolae* was investigated using immunoblotting, immunofluorescence and phosphatase activity.

3- Results also shown the expression of SAP1Cyc18 protein in *Leishmania tarentolae* based on phosphatase activity.

Future work:

Evaluate the protective immune response of the filamentous proteins against toxoplasmosis in a mouse model.

Parasite ecology

ECO-P-01

Malaria and intestinal parasites in pregnant woman at Abobo district (Abidjan, Côte dIvoire)

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Question: A prospective study was carried out from 2010 to 2012 at the Hôpital Général d"Abobo (HGA) in Abidjan, in order to determine the impact of infectious and parasitic diseases on child cognitive development.
Methods: Blood samples were examined by means of thick drop and blood smear; as for stool by direct examination and concentration by formalin-ether method. We evaluated the prevalence, the parasite load of malaria and gastrointestinal parasites; then we investigated the risk factors for these disorders.
Results: Overall, 331 pregnant women in the last trimester of their pregnancy were enrolled. The plasmodic index was 3.9% with infestation specific rates of P. falciparum from 100%. Concerning digestive protozoa, it has been observed 71.3% of nonpathogenic, against 9.7 % of pathogens, either an overall prevalence of 51.4% of digestive parasites. The calculated average parasitic loads revealed 3089.2 tpz/µl of blood (95 % CI: 591.1-5587.3) for malaria, 6.5 eggs per gram of stool (95 % CI: 0.4-13.4) for intestinal helminths and one parasite by microscopic field for protozoa (common infestation). It has been shown that the occurrence of malaria has been linked to the non-use of impregnated mosquito nets (x2 = 0.012; p = 0.018), not to age. No link could be established between the presence of digestive parasites and the age of pregnant women, or socioeconomic conditions (level of education, profession, type of toilet).
Conclusions: Malaria is less common in pregnant women while the rate of digestive parasites remains high.

ECO-P-02

Development of a biological tick trap based on attract-and-kill strategy – Screening of attractants

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In Europe, *Ixodes ricinus* (Acari: Ixodidae) is the most abundant tick species and the main vector of tick-borne diseases (TBD). Increased public awareness concerning TBD has raised interest in an effective tick control. The aim of this project is to develop a control method against *I. ricinus* based on an attract-and-kill strategy. Therefore, we screened long and short range attractants as well as

substances causing arrestment in ticks. In behavioural assays using a novel y-olfactometer we performed a broad screening of compounds of the classes of aldehydes, lactones, terpenoids, and others for their attractivity towards *I. ricinus* nymphs. Here we demonstrate a significantly attractive effect of CO2 and acetone on the tick. Further we screened substances for their potential to induce arrestment in *I. ricinus* using a static olfactometer bioassay, where ticks choose between four filter papers for arrestment. Here, we detected compounds of the classes of purines and their derivatives with arrestment activity. These attractants will be released through a capsule-based biopolymer system, that is coated with tick arrestment stimuli and coupled with an entomopathogenic fungus (Metarhizium spp.) to kill the tick. Future research should investigate whether similar results are obtained when adult *Ixodes* ticks or other tick species are tested.

Parasite-host interactions

PHI-P-01

***Eimeria bovis* macromeront formation in endothelial host cells: Role of sterol uptake and transport**

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Introduction: *Eimeria bovis* macromeront formation in bovine endothelial host cells is a highly cholesterol-demanding process. Since apicomplexans are general considered as defective in cholesterol synthesis, they have to scavenge cholesterol from the host cell by either enhancing the uptake of extracellular cholesterol sources or by up-regulating the host cell *de novo* biosynthesis. We recently showed that uptake of low density lipoproteins (LDL) from extracellular sources is enhanced in *E. bovis*-infected cells and boosts parasite development.
Objective: We here examined the effects of different inhibitors that either block exogenous sterol uptake or intracellular transport on the formation of *E. bovis* macromeronts.
Material and Methods: From day 1 and 10 p. i. onwards, *E. bovis*-infected endothelial host cells were treated with inhibitors of sterol uptake (sucrose, dextransulfate, poly-I, poly-C, ezetimibe) and of intracellular sterol transport and release from endosomes (progesterone, U18666A). As read-out for inhibitor effects, the size and number of macromeronts and merozoite I production were estimated at days 19 and 24 p. i., respectively.
Results: Specific inhibition of sterol uptake via ezetimibe led to a highly significant blockage of macromeront formation and total abrogation of merozoite I production proving the relevance of sterol uptake for parasite development. Less prominent effects were induced by the non-specific blockage of LDL internalization via sucrose, poly-I and poly-C. In addition, the blockage of cholesterol transport from exosomes via progesterone and U18666A treatments also resulted in significantly decreased numbers and sizes of macromeronts and merozoite I production.
Conclusion: The current data underline the relevance of exogenous sterol uptake and intracellular cholesterol transport for adequate *E. bovis* macromeront development.

PHI-P-02

Genome-wide association study of endoparasite resistance in a population of Black and White dairy cows

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Infections with endoparasites cause high economic losses in pasture-based dairy industry. Quantitative-genetic studies have shown that host resistance to gastrointestinal nematodes (GIN) has a considerable genetic component. Genome-wide association studies (GWAS) using dense single nucleotide polymorphism (SNP) marker panels enable the identification of the underlying genomic architecture of parasite traits. The objectives of the present study were i) to identify SNP markers and candidate genes associated with resistance to endoparasite infections in dairy cows; ii) to assess SNP effect correlations between endoparasite traits within identified genomic regions of potential physiological significance. Faecal samples were collected twice from 1166 cows and assessed for GIN, the bovine lungworm (*Dictyocaulus viviparus*) and the liver fluke (*Fasciola hepatica*). Blood samples were collected from 148 of these cows, which were genotyped using the BovineSNP50 Bead Chip and subsequently imputed to Illumina using a multi-breed reference panel of 2188 animals. The dataset contained 423,654 SNPs. Endoparasite traits (faecal egg/larvae counts) were precorrected for fixed effects via linear mixed models using the dataset of 1166 cows. Precorrected phenotypes for the 148 genotyped cows were subsequently used in the GWAS. For GIN, 7 candidate genes were identified, located on bovine chromosome (BTA) 2, 4, 6, 18, 22, 24, while 32 candidate genes were found for *D. viviparus*. For *F. hepatica*, 4 candidate genes were detected on BTA 1, 4, 15 and 17, including one gene (ALCAM) which is involved in immune response mechanisms. High SNP effect correlations (≥ 0.95) for endoparasite traits in two genomic regions on BTA13 associated with *D. viviparus* indicate a partly joint genetic basis for host resistance. The findings extend the understanding of the genetic architecture of bovine host resistance to helminth infections.

PHI-P-03

Identification of *Plasmodium falciparum* erythrocyte membrane protein 1 (*Pf*EMP1) molecules involved in the interaction with various human endothelial receptors

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Plasmodium falciparum infected erythrocytes (IEs) evade the human immune reaction due to their adhesion to the human vascular endothelium. Every single parasite has 60 unique *var*-genes, coding for membrane-bound *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP1), expressed during the asexual intraerythrocytic mature phases of the parasite. Specific *Pf*EMP1 domains are described as key factors of adhesion.

By now, 23 human receptors are known to bind IEs but only for 7 of them *Pf*EMP1 is identified as the parasite ligand. We focus on several *P. falciparum* culture- and field isolates to determine putative

Static binding assays: define initial *Pf*EMP1 binding abilities towards heterologous expressed receptors by use of transgenic self-engineered CHO cells
Transcriptome profiles: IEs, enriched for specific binding towards selected receptors were analyzed regarding differential gene expression via mRNA-Seq
Transmission electron microscopy: quantitative and comparative analysis of morphology, appearance and versatility of different *P. falciparum* isolates from *ex vivo* until long-term cultivation

For human receptors P-selectin and CD9, differentially expressed *var*-genes were specifically described (Metwally & Tilly et al. 2017). Furthermore, IEs populations were enriched and selected for 4 (un)known receptors. Their transcriptome will be analyzed to validate the initial putative *Pf*EMP1 interaction and to reveal the decisive *var*-genes.
Comprehensive genetic and phenotypic characterizations of IEs enriched for the binding to specific human receptors will expand our understanding of *P. falciparum* adhesive interaction and may finally lead to development of mechanisms for parasitic inhibition.

This work is supported by the Joachim Herz Stiftung and PIER Helmholtz Graduate School.

PHI-P-04
The importance of *Plasmodium vivax* VIR proteins for the cytoadhesion of infected erythrocytes

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Introduction: While present in the blood *P. vivax* invades reticulocytes. Although all stages of *P. vivax* are present in the blood the total biomass does not correlate with the amount of circulating parasites. This means the parasite has to adhere to the receptors of the endothelial, called cytoadhesion. It is postulated that some members of the so-called VIR protein family mediate the adhesion.
Objectives: In this study, we are investigating different VIR proteins, their localisation in the infected erythrocytes (IEs) and their binding properties to specific surface receptors.
Materials & Methods: VIR proteins with a predicted transmembrane domain and/or an export motive are codon optimized to *P. falciparum*, fused with either a GFP- or a 3x HA-Tag and transfected into this parasite. The localisation is detected by an immunofluorescence assay (IFA) and the binding capacity to certain receptors is studied under static and flow conditions.
Results: The results of the IFAs show a different distribution among the IEs. For some VIR proteins, co-localisation with Maurer's-Clefts proteins has been demonstrated, and other VIR proteins co-localise with proteins of the knob region. In addition, the localisation of one protein changes between the trophozoite stage and the schizont stage.
Conclusion: The proteins investigated so far have all been exported from the parasite. But it is not possible to predict the localisation within the IE. If the to the membrane exported proteins bind to the tested receptors, we have evidence that they are part of the cytoadhesion of *P. vivax.*

PHI-P-05
Development of tsetse fly-transmitted African trypanosomes in human skin tissue models

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Introduction: African trypanosomes are a major threat to health and the economy in large parts of sub-Saharan Africa. The unicellular parasites not only cause the deadly human sleeping sickness but also the widespread disease nagana in cattle. Trypanosomes have attracted the attention of modern biology for many decades. Major discoveries in molecular cell biology and biochemistry have been made using trypanosomes as a model. Despite this, significant parts of the trypanosome life cycle and many aspects of the human infection remain enigmatic, including the role of mammalian skin as the first site of infection.
Objectives: We want to investigate the early course of infection of tsetse fly-transmitted African trypanosomes in mammalian skin.
Materials & Methods: Experimental approaches to host skin as first site of infection have been obstructed by the lack of appropriate animal models and vector transmission differs significantly from experimental syringe passage. Therefore, we employ primary human skin models and use tsetse flies to infect the skin models with trypanosomes in the most natural way.
Results: We have generated and characterized an advanced primary human skin model consisting of an epidermal and dermal component that resembles native human skin. As proof-of-principle we have shown that infected tsetse flies probe on engineered human skin equivalents, thereby injecting viable infective metacyclic trypanosomes into the skin tissue. Moreover, we could show that the injected parasites start to proliferate and persist in the skin model for several days.
Conclusion: Our developed human skin model could be infected successfully with trypanosomes by the tsetse fly and we were able to detect living and dividing trypanosomes in the artificial skin tissue. Endothelialisation of the skin model will allow us in the future to study, in addition to the development of the parasites in the skin, the transmigration of the parasites into the circulatory system.

PHI-P-06
RNAi-based trigger gene silencing approach in *Entamoeba histolytica* to identify pathogenicity factors involved in amoebic liver abscess formation

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Introduction: Recently, we identified *E. histolytica* clones, which differ in their pathogenicity. Clone $B2^{p}$ is able to induce amoebic liver abscess (ALA) formation in the animal model. In contrast, clone $B8^{np}$, which derived from the same cell line (HM-1:IMSS-B) is non-pathogenic. In comparison to clone $B2^{p}$, clone $A1^{np}$ (derived from cell line HM-1:IMSS-A) induces smaller abscesses, which regenerate faster. Based on comprehensive transcriptome studies, we identified a set of genes that are differentially expressed. Using ectopic overexpression of 20 candidate genes, 8 highly expressed genes

were identified to correlate with a reduced pathogenicity and only one that increases pathogenicity to a certain extend.
Objectives: To analyse the influence of these candidate genes in more detail a RNA interference trigger mediated gene silencing approach was used and 13 silencing transfectants were selected for mice infection experiments.
Material and Methods: We identified trigger sequences that specifically allow gene silencing in the A and B clones, respectively. The silencing was confirmed by qPCR and the ALA formation was assessed after intrahepatically injection of the silenced amoeba in mice.
Results: We were able to silence 15 out of 25 investigated genes and interestingly, the silencing is stable also without addition of the selectable marker. An effect on ALA formation was seen after silencing the genes EHI_026360 (putative phosphoserine aminotransferase) and EHI_180390 (putative AIG1 family protein), where silencing in clone $B8^{np}$ increased pathogenicity and after silencing of EHI_127670 (hypothetical protein) where silencing in clone $B2^{p}$ decreased pathogenicity.
Conclusion: This novel RNAi-based trigger gene silencing approach is a useful tool to investigate putative pathogenicity factors of *E. histolyica*.

PHI-P-07
Carrion crows (*Corvus corone*) of southwest Germany: important hosts for haemosporidian parasites

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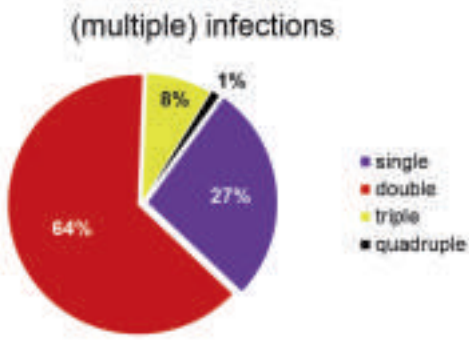
Question: Avian malaria parasites (*Plasmodium* spp.) and other Haemosporida (*Haemoproteus* and *Leucocytozoon* spp.) form a diverse group of vector-transmitted blood parasites that are abundant in many bird families. Recent studies have suggested that corvids may be an important host for *Plasmodium* spp. and *Leucocytozoon* spp.
Methods: To investigate the diversity of Haemosporida of resident Carrion Crows (*Corvus corone*) and Eurasian Magpies (*Pica pica*) in southwest Germany, 100 liver samples of corvids were examined using a nested PCR method to amplify a 1063 bp fragment of the haemosporidian mitochondrial cytochrome b gene. The phylogenetic relationship of parasite lineages obtained from these birds was inferred.
Results: Haemosporidian DNA was detected in 85 Carrion Crows (89.5%) and in all five Eurasian Magpies. The most abundant parasite genus was *Leucocytozoon* with a prevalence of 85.3% (n = 95). 65.3% of the samples (n = 62) contained multiple infections (see Fig. 1). Thirteen haemosporidian lineages were isolated from the corvid samples. Female Carrion Crows were more likely infected with haemosporidian parasites than males.
Discussion: This study provides the first insight into the diversity of haemosporidian parasites of corvids in Germany. Very high prevalences were found and based on the applied diagnostic method also a high amount of multiple infections could be detected. Due to the high diversity of haemosporidian parasites found in corvids, they seem to be excellent model organisms to test species deliminations in haemosporidian parasites.

Figure 1



PHI-P-08

Avian malaria on Madagascar: Specialization of endemic haemosporidian parasites

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Question: The island of Madagascar is located approximately 400 km east of Africa in the Indian Ocean. Due to its isolation from mainland India and Africa it has many endemic species and is classified as an important biodiversity hotspot. Avian Malaria is caused by haemosporidian parasites including the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. To date, few studies exist on blood parasites of Malagasy birds and there is no information about their degree of specialization on their bird hosts.

Methods: Over 1000 blood samples of Malagasy birds of over 50 different species (mainly Passeriformes), sampled in the years 2003 – 2016, were analyzed using molecular techniques. With a newly established nested PCR a large fragment of the parasites`cytochrome b gene was amplified. It was determined if the parasite lineages detected might be specialized on a bird host or not.

Results: We found an extremely high variety of blood parasites. Over 80 different lineages of haemosporidian parasites could be identified. Due to the high number of investigated birds we could in some cases determine if the parasite lineage found is a specialist or a generalist on its bird host.

Conclusion: Most of the detected *Plasmodium* lineages seem to be generalists, whereas all *Haemoproteus* lineages might be either specialists on a single bird species or a genus. For *Leucocytozoon* spp. it seems like there is both - specialists and generalists. More large scale studies are needed to vaify the degree of specialisation especially for *Leucocytozoon* spp.

PHI-P-09
Drug-induced clearance of helminth infection restores efficacy of anti-Influenza vaccination.

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Introduction: Worldwide 2 billion people are affected by infections with parasitic helminths. Thereby helminths delay their immune driven eradication by suppressing the host's immune system. Hence, immune responses against unrelated antigens are suppressed as well. We have shown previously that antibody (Ab) responses to anti-Influenza vaccination were inhibited in mice being simultaneously infected with the parasitic nematode *Litomosoides sigmodontis*. Strikingly, even if vaccination was performed several months after natural, immune driven clearance of the parasite, vaccination efficacy was still impaired.
Objectives: We intend to investigate if drug-induced deworming is capable to restore Ab responses to anti-Influenza vaccination.
Material and Methods: Mice were naturally infected with *L. sigmodontis* and infection was terminated at defined time points by Flubendazol (FBZ) treatment. Mice were vaccinated with the seasonal (16/17) anti-Influenza vaccine Begripal (Sequirus). HA-specific Ab responses were quantified by ELISA and the functional hemagglutination inhibition assay (HI).
Results: FBZ treatment was found to effectively clear helminth infection in fully susceptible BALB/c mice and in semi-susceptible C57BL/6 mice. *L. sigmodontis*-infected, untreated mice displayed reduced Ab response to anti-Influenza vaccination compared to non-infected mice. *L. sigmodontis*-infected, FBZ treated mice displayed similar Ab response as naïve mice. The mechanism underlying the different outcome of drug-induced and immune driven clearance is subject of current research.
Conclusion: Our results suggest that drug-induced deworming abrogates the helminth-induced suppression that is still observed several months after immune driven, natural clearance of helminth infection.

PHI-P-10
The Stubborn Apicomplexan: Lack of Transcriptional Plasticity to Host Immune Defenses

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Parasites can respond plastically or in a genetically programmed (or hard-wired) fashion to environmental changes such as host immune responses to infection.
We assumed that such functional plasticity would be discernible in the parasite transcriptome and set out to test whether an apicomplexan parasite, *Eimeria falciformis*, behaves plastically at the transcriptional level.
We performed dual RNA-seq on the life cycles of *E. falciformis* in its natural host, the mouse, and of sporozoite and oocyst stages. Mice with different immune status were used in order to evaluate the parasite"s plastic responses. Host transcriptomes were also analyzed.

Parasite and host transcriptomes were compared between naïve and challenge infected mice, as well as between immune competent and immunodeficient ones. These different mice show transcriptional differences as well as differences in parasite reproduction (oocyst shedding). Differently abundant host genes, such as TGFβ, EGF, TNF and IL-1 and IL-6, indicate enrichments for immune reaction and tissue repair functions during infection but display different profiles depending on the immune competence of the mice. Much in contrast, parasite transcriptomes were not different between *E. falciformis* in differently immune competent mice. Instead, parasite transcriptomes have distinct profiles early and late in infection independently of mouse immune status. Parasite transcriptomes are characterized largely by gene groups enriched for annotations for biosynthesis (early) or motility (late). Extracellular sporozoite and oocyst stages also showed distinct transcriptional profiles.
We propose that the niche and host-specific apicomplexan parasite *E. falciformis* uses a genetically canalized program of infection. This might limit the potential of the parasite to adapt to new host species or niches, forcing it to coevolve with its host.

PHI-P-11
Characterizing protein function at the parasite - host interface during blood stage infections of *Plasmodium berghei*.

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Plasmodium remodels the cell membranes of its hosts in order to control the interface between host and parasite. The generation of membrane structures in the cytoplasm of infected red blood cells is a feature shared across Plasmodium species. While these structures appear necessary for Plasmodium growth within red blood cells, the functions of the parasite proteins known to localize to these membranes are largely unknown. In P. berghei-infected red blood cells, membrane structures called intra-erythrocytic P. berghei-induced structures (IBIS) are labeled with the exported protein IBIS1. This protein localizes to IBIS in blood stages and to the parasitophorous vacuole membrane (PVM) and tubovesicular network in liver stages of infection. Through co-immunoprecipitation of IBIS1, we identified two additional exported proteins, IBIS2 and IBIS3. Like IBIS1, they too are expressed in both liver and blood infection stages, and they colocalize with IBIS1 in the liver-stage PVM. The presence of IBIS2 and IBIS3 across several Plasmodium species and multiple developmental stages indicates that parasite-modified membranes in host cells may serve conserved functions important for Plasmodium intracellular residence.

PHI-P-12

***Wolbachia pipientis* in natural populations of mosquito vectors of *Dirofilaria* from Russia**

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This study is focused on bloodsucking mosquitoes from European part of Russian Federation. We have screened Culicids vectors possibly involved in *Dirofilaria* transmission for infection with *Wolbachia pipientis*.

Materials & Methods: In total, about 3000 adult female mosquitoes from 20 different species were captured during 2012–2017. All captured mosquitoes were analysed by morphology, in some cases by DNA analysis. Detection of *Dirofilaria* was done by PCR using specific primers for amplifying ITS2 region of rRNA and *COI* gene. Detection of *W. pipientis* was performed by PCR with specific primers for *Wolbachia surface protein* gene (*wsp*).
Results: Eleven mosquito species from *Ochlerotatus, Anopheles, Aedes* and *Culex* genera were found to be infected with *Dirofilaria* spp. The presence of *W. pipientis* was found in the 6 mosquito species. Since, it is known that *W. pipientis* protects mosquitoes from infections with arboviruses and parasites, we investigated a possible association between the occurrence of *W. pipientis* and the development of the *D. immitis* not only in pooled abdomens, but also in the head-thorax of individual specimens.
Conclusion: Our results showed that *W. pipientis* does not prevent the ingestion of *Dirofilaria* by mosquitoes in nature. But it cannot be ruled out that the bacterium influences the larvae development of Dirofilariae up to infective stage in mosquitoes.

This work was supported by the Russian Foundation of Fundamental Research, grants N 16-04-00091.

PHI-P-13

Deciphering *P. berghei* EXP-1 function during pre-erythrocytic and erythrocytic development at the host-parasite interface

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In malaria infection, the liver represents the first site for pathogen replication, resulting in several thousand progenies originating from one single parasite. This extraordinary expansion phase of the plasmodial parasite is not associated with clinical symptoms. *Plasmodium* parasite invasion of hepatocytes is accompanied by the formation of a parasitophorous vacuole (PV) representing a niche

for further development. During the process of intra-hepatic growth, the PV membrane (PVM) is remodeled by the parasite, including the incorporation of plasmodial proteins.
The cross-stage protein Exported protein 1 (EXP-1) represents the first described PVM-resident protein in both intrahepatic and intra-erythrocytic life-cycle stages of the parasite. Even though EXP-1 was discovered more than 30 years ago and has been considered a potential vaccine candidate, the function of EXP-1 remains still enigmatic.
Previously, it has been suggested that EXP-1 functions as a glutathione S-transferase during intra-erythrocytic development. Recent data moreover demonstrated that the outermost C-terminal domain of EXP-1 is involved in the recruitment of the host-cell factor Apolipoprotein H (ApoH) during liver-stage development, thereby facilitating successful progression.
To further decipher the functional role of EXP-1 we generated *P. berghei* lines harbouring specific mutations of EXP-1. Characterization of those lines suggested that EXP-1 might exert different functions during the parasite life cycle. Interestingly, we observed that one of our mutant lines was severely impaired in intra-hepatic development *in vitro* and *in vivo.* In the context of future studies we will now utilise a multi-facetted approach to receive a more detailed understanding of EXP-1 functionality at the host-parasite interface.

PHI-P-14

"New king new law": Biting midges as a probable vector of the etiologic agent of cutaneous leishmaniasis in Ghana.

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Introduction: Cutaneous leishmaniasis (CL) has been on the spotlight due to the efflux of refugees from Syria to non-endemic. New vector is anticipated in emerging species of *Leishmania* in *Leishmania enriettii* complex, evidenced in previous works and recent laboratory procedures. *Leishmania* species responsible for cutaneous leishmaniasis in Ghana is yet-to-be names. The vector and the reservoir hosts are still unknown despite several sandflies investigated in the endemic area.
Aim: This work seeks to incriminate biting midges prevalent in the endemic area as a probable vector after this laboratory study.
Material and Methods: Two vectors *Lutzomyia longipalpis* and *Culicoides sonorensis* were infected with the newly isolated *Leishmania* from Ghana, under the same conditions and kept for more than 10 days. The vectors were dissected daily to study the infections establishment in the vectors from day 1 to 10.
Results: Heavy infections characterised both vectors at the blood meal stage, until the blood meal were fully digested. Infections in *Lu*. *longipalpis* decreased to 0% 3 days after bloodmeal digestion. Infections in the *C*. *soronensis* were retained beyond 10 days to ≈80%, colonising the midgut and the stomodeal valve.
Conclusion: The human pathogenic *Leishmania* responsible for CL in Ghana, heavily infected *Culicoides sonorensis*, colonizing the midgut and stomodeal valve up to and beyond day 10 at higher infectivity rate. This supports the idea that midges could be vectors of this new species of *Leishmania* in Ghana and successfully transmit the parasite. Since midges are repetitive feeders, a second blood meal could

help the parasite to probably survive longer and trigger molecules that might help *Leishmania* develop to the metacyclic promastigotes. Moreover, the mesh-gelatinous-like appearance somewhat around the parasite in the midgut of the *Culicoides* requires further investigation. The morphological structure and molecular identity of midges in the endemic sites need to be studied.
Ongoing work: Few field trips have been made and ≈300 biting midges collected in storage. Morphological and molecular identification are underway to implicate or otherwise, the midges as the vector of the new species of *Leishmania* responsible for CL in Ghana.

PHI-P-15
Cross-species correlation of host and parasite gene expression as a tool to identify protein interactions between Plasmodium and hosts

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The analysis of complete transcriptomes has increased our understanding of how individual organisms respond to environmental stimuli. Additionally, correlated expression of genes within one species has helped to identify complexes of physically interacting proteins.
A wealth of studies on Plasmodium sp. infections have either tried to understand host or parasite transcriptomes and deposited sequencing reads to databases. "Contamination" in studies focussed on one organism - the host or the parasite - often allows us to obtain gene expression data of both organisms.
Here we present our collection of experiments that provide RNA-Seq data on Plasmodium-host interactions and use this data to study their co-regulated genes. A total of 4870 sequencing runs were included in our study coming from 43 publicly available datasets. This data comes from 8 different sequencing platforms and also from different host organisms and parasites: Plasmodium falciparum and P. vivax in humans, P. berghei and P. chabaudi in mice as well as P. coatneyi, P. cynomolgi and P. knowlesi in macaques. We show how the use of orthologs across host and parasite species can help to improve predictions and to infer crucial interactions with strong evolutionary conservation.
We discuss how machine learning algorithms can be designed and trained with existing protein-protein interactions to predict possible and previously unknown interactions between host and parasite proteins. From a broader perspective, our project showcases how the immense growth of biological information can be harnessed for parasitology in silico.

Parasitic helminths

PHE-P-01

Experimental Study on Life Cycle of *Hypoderaeum conoideum* (Block, 1872) Diez, 1909 (Trematoda: Echinostomatidae) Parasite from the North of Iran

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Introduction: Human Echinostomiasis is an intestinal disease caused by the members of family Echinostomatidae parasites. Although. *Hypoderaeum conoideum* is a common parasite that has been reported in many studies in the world, but no research has been found that surveyed the development of cercaria to adult stages in Iran.
Objectives: The aim of the present research was to identify echinostomatidae cercariae emitted by *Lymnaea palustris* snails from Mazandaran province in the North of Iran based on the morphological and morphometrical characteristics of the different stages of the parasite life cycle in an experimental study.
Materials & Methods: Echinostomatidae cercariae were collected from *L. palustris* (Gastropoda: Lymnaeidae) of the North of Iran. To collect metacercaria, 50 healthy snails were infected with cercariae experimentally (50 cercariae for each). To obtain the adult stage, 9 laboratory animals (3 ducks, 2 rats, 2 mice and 2 quails) were fed with 60 metacercaria for each. To identify parasites, the different stages of worm were examined using light microscope and then the figures were drawn under the camera Lucida microscope and measures were determined.
Results: Averagely, 15 metacercaria were obtained from each snail that had been previously exposed with cercariae. Ducks presented worm eggs in feces after 10-15 days post-infection. Intestinal worms were collected and identified as *H. conoideum* on the bases of the figures and measures of cephalic collar, the number of collar spine, suckers diameter ratio, testes arrangement, etc.
Conclusion: *H. conoideum* cercariae and adult worm are described. This is the first report of the different stages of the life cycle of this parasite in Iran.

PHE-P-02

The Feasibility of a Re-mapping Protocol for Lymphatic Filariasis in Areas where Transmission is Uncertain in Ethiopia

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Lymphatic filariasis (LF) is one of the world"s leading causes of permanent disability. WHO proposed a comprehensive strategy to eliminate LF by 2020, interruption of transmission through MDA and morbidity management. The global program to eliminate lymphatic filariasis recommends mapping as an initial step to determine the need of MDA in Implementation units (IUs). The existing WHO guideline recommends two sites per IU for mapping and a sample of 100 adults should be tested for

antigenaemia. In low transmission setting this strategy has limitations. An alternative mapping method that could resolve this situation is useful. This study was, therefore, designed to assess the Lf endemicity level of IUs in Ethiopia which have low transmission setting. The 2013 mapping in Ethiopia resulted in 45 IUs with antigenemia result not enough for programmatic decision making. To solve this gap the school based mapping was conducted in two phases; phase I was conducted in 8 IUs, to evaluate the protocol itself. In this survey, schools were selected by either systematic or cluster sampling, based on the number of schools in the IUs. From each selected school children of grade 4 to 8 were sampled systematically and tested. The number of positive result was compared against the critical value. From 41 IUs involved in second phase re-mapping survey, antigenesimia was tested from 16,365 children of target grades in selected schools. In 39 of the IUs, the number of antigen positive identified in the re-mapping surveys was below the critical cut off, suggesting transmission of LF is not ongoing in these IUs. Where as in two of the IUs, the number of antigen positive identified was above the critical value suggesting that the LF transmission is ongoing. In low prevalence areas, where the current WHO protocol has several limitations, this re-mapping study design will provide enough information on the LF transmission situation which may help programs to make evidence based decision.

PHE-P-03
***Strongyloides stercoralis* in humans and dogs – A study in northern Cambodia**

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Introduction: Strongyloidiasis is a neglected soil born helminthiasis caused by the nematode *Strongyloides stercoralis*. Estimates of the number of people infected with *S. stercoralis* go up to 370 million worldwide. *S. stercoralis* infection frequently remains asymptomatic, but it can last for many years and develops into life-threatening forms if the carrier becomes immuno-compromised. There has been a long-lasting debate whether *Stronglyoides sp.* naturally occurring in dogs are *S. stercoralis* and dogs therefore are a possible source of zoonotic transmission of *S. stercoralis*. Although free-living generation of *S. stercoralis* has females and males it has been a matter of debate if the they reproduce sexually or by sperm dependent parthenogenesis.
Objective: 1) Are dogs a source for zoonotic transmission of strongyloidiasis and 2) Do free-living *S. stercoralis* reproduce sexually or parthenogenetically.
Materials and Methods: 1) We isolated *Strongyloides sp.* from humans and their dogs in rural northern Cambodia, a region with a high incidence of strongyloidiasis. We determined parts of the

nuclear 18S rDNA and the mitochondrial *cox*1 sequences from individual worms and for selected worms we sequenced the whole genome. 2) We performed crossing experiments in the laboratory and isolated mothers and their progeny from the wild. We genotyped these parents and their progeny at molecular genetic markers.

Results: 1) In dogs there exist at least two genetically separated populations of *Strongyloides*. One population appears to be shared with humans, the other is restricted to dogs. 2) The progeny of free-living adults inherit genetic markers from both parents in a Mendelian manner.

Conclusion: 1) There is strong potential of dogs as a reservoir for zoonotic transmission, suggesting dogs should be considered for measures for improved hygienic conditions and should be treated along with humans. 2) The free-living generation of *S. stercoralis* reproduces sexually.

Figure 1

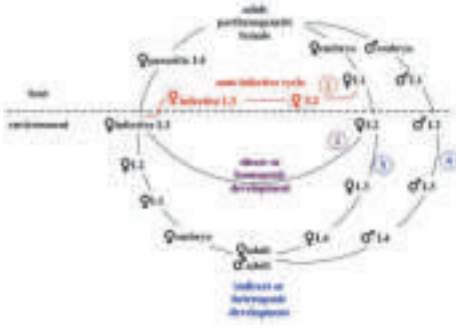
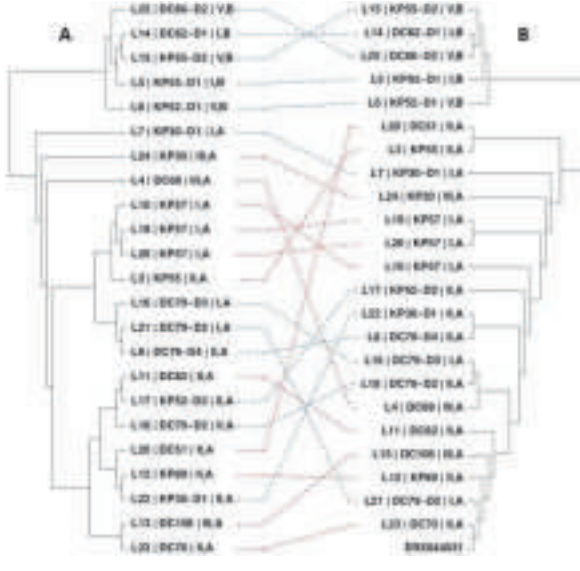


Figure 2



PHE-P-04

Epizootiology of Fasciolosis in sheep (Ovis Aries) raising in geoclimatic setting of Poonch District of Azad Kashmir, Pakistan

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Fasciolosis is a wide spread helminth parasitic disease of herbivore outcomes in economic losses in livestock appearing in the form of mortalities, infected liver, slow growth and reduction of milk, meat and wool production. This study was designed to find out the prevalence of ovine fasciolosis in and around Poonch District of Azad Kashmir. For this purpose, a total of 300 sheep of various age groups and of both genders were randomly selected. Faecal samples were collected rectally hygienically in a sterile bottle and processed through conventional sedimentation technique in the Laboratory of Parasitology, Department of Pathobiology, Faculty of Veterinary and Animal Sciences, The University of Poonch, Rawalakot Azad Kashmir, Pakistan. Out of these, 101(33.6%) sheep were found positive for Fasciolosis through coprological examination. Results revealed that prevalence of fasciolosis was more 34.6% in ewe than 30.6% in ram. There was no significant difference (P>0.05) in prevalence of Fasciolosis based on gender of sheep. However, there was significant difference (P

PHE-P-05

***Schistosoma mansoni* histone deacetylase 8 (*Sm*HDAC8) interacts with the Rho GTPase *Sm*Rho1.**

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Introduction: Histone deacetylase 8 of *Schistosoma mansoni* is a privileged target for drug discovery. Invalidation of its transcription by RNAi led to impaired survival of the worms in infected mice and its inhibition causes apoptosis and death. The precise biological roles of *Sm*HDAC8 are unknown and to determine why it is a therapeutic target the study the cellular signaling pathways involving this enzyme is essential. Protein partners of *Sm*HDAC8 have been characterized by screening a yeast two-hybrid (Y2H) cDNA library and mass spectrometry (MS) analysis. Among these partners we identified *Sm*Rho1, the schistosome orthologue of the human RhoA GTPase, which is involved in the regulation of the cytoskeleton.
Objective: To characterize the interaction between *Sm*HDAC8 and *Sm*Rho1 and the role of *Sm*HDAC8 in cytoskeletal regulation.
Materials & Methods: To confirm and study the interaction between *Sm*HDAC8 and *Sm*Rho1 we performed Co-IP experiments and two heterologous expression systems (Y2H assay and *Xenopus oocytes*). MS analysis identified *Sm*Rho1 acetylation. The role of *Sm*HDAC8 in cytoskeleton organization was studied by treating adult worms with a selective inhibitor.
Results: We identified two isoforms of *Sm*Rho1, *Sm*Rho1.1 and *Sm*Rho1.2 and demonstrated a specific interaction between *Sm*HDAC8 and the *Sm*Rho1.1 isoform involving its C-terminal moiety. We have

also shown that *Sm*Rho1 is acetylated on lysine K136. Treatment with an *Sm*HDAC8 inhibitor caused massive disruption of the worm cytoskeleton.
Conclusion: Our results show that *Sm*HDAC8 is potentially involved in cytoskeleton organization via its interaction with the *Sm*Rho1.1 isoform.

PHE-P-06
***Schistosoma mansoni* infection among preschool-aged children on Ijinga Island, Northwest Tanzania: prevalence and intensity of infection**

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Introduction: Recent evidence indicates that schistosome infections are already acquired in early childhood in highly endemic areas. In Tanzania most studies focuses on school-age children and detailed epidemiological information on *S. mansoni* infection among preschool-aged children (PSAC) is lacking. In addition, in these settings, the most used diagnostic test for schistosomiasis is the Kato-Katz (KK) method. However, it shows a low sensitivity in detecting low-infection intensity, which are commonly seen in young children. A promising method to diagnose *S. mansoni* infections, even in PSAC, is the urine-based POC-CCA cassette test.
Objectives: This study was conducted to determine the prevalence and intensity of *S. mansoni* infection among PSAC on Ijinga Island, Northwest Tanzania.
Materials & Methods: A community based cross sectional study was conducted in September 2016. Ninety-nine PSAC (aged 1- 6 years) were included in the study and submitted a single stool and urine samples. The KK technique and the POC-CCA test were used to diagnose *S. mansoni* infections.
Results: The mean age of PSAC was 5.08 years (±0.83 years SD). Based on the KK method, 62.6 % PSAC were infected with S. mansoni. Most infections were light (n = 34; 34.3 %), whereas 20 children (20.2 %) had a moderate (100–399 eggs per gram of faeces (epg)) and eight children (8.1 %) had a heavy infection (≥400 epg). A single POC-CCA test identified 94 (94.9 %) children having active schistosome infection. A statistically significant association was observed between prevalence of S. mansoni and age ($P<0.01$).
Conclusion: Our results showed an alarmingly high number of S. mansoni infected PSAC. Current control programs focus on the school-aged population, and hence a considerable number of infected children might be restrained from treatment. Our study shows further that POC-CCA test detects more children infected with *S. mansoni* than the widely used KK technique.

PHE-P-07

Identification of helminths in house mice from the European Hybrid Zone: Combining classic taxonomy and a molecular approach

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Parasitemia in European house mouse hybrid zone has been proposed as a potential ecological isolating mechanism between two subspecies of the house mouse. In order to assess parasitemia and to deduce potential fitness effects on the hosts, correct taxonomical identification of helminths is warranted. However, only few studies on the taxonomy of helminths of house mice have been published and provide neither precise morphological identifications nor molecular data for automated identification based on DNA sequence comparisons.
For that reason, this study aims to describe the diversity of helminths in house mice from the European Hybrid Zone (Brandenburg, Germany 2016 – 2017). We use an approach based on microscopical observation (optical and electron microscopy) and identification based on comparison to available nucleotide sequences for fragments of mitochondrial (COI) and nuclear (18S) marker genes.
We identified 10 helminth species in a total of 435 house mice. The well described *Syphacia obvelata, Aspiculuris tetraptera*, *Trichuris muris* and *Heterakis spumosa* were observed with high prevalences of 45,5%, 15,4%, 10,8 and 6,4%, respectively. We were able to distinguish the cestodes *Hymenolepis microstoma*, *H. diminuta* as well as *Taenia martis* and *T. taeniformis,* which exhibit a lower prevalence (< 3,5%) and were previously not identified at species level in research on house mice. We present a description of the morphology of *Catenotaenia pusilla* and *Mastophorus muris* at a level of detail not previously available.
In contrast to previous studies based on microscopic examinations during field sampling only, our observations allow us to conduct re-descriptions for morphological taxonomy. Identifications were confirmed with DNA sequence data, also allowing to link future observations to our enhanced descriptions. Thus, this work allows quantitative assessments of parasitemia in the house mouse hybrid zone at an unprecedented level of detail.

PHE-P-08

Iatrogenic helminth infection in a patient with systemic lupus erythematodes under hydroxychloroquine therapy: a case report.

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Case presentation: A 27-year-old German lady was referred for workup of various food intolerances, diffuse abdominal complaints including stool irregularities and flushing. She had been diagnosed with autoimmune hypophysitis three years before which later was diagnosed as a possible manifestation of

systemic lupus erythematodes. Other comorbidities were joint hypermobility (suspicious of Ehlers-Danlos Syndrome) and abnormalities of the coagulation system (von Willebrands disease, heterozygote factor V / H1299R and MTHFR / C667T mutation with normal serum homocystein levels. The patient was treated with antihistaminics and hydroxychloroquine. In addition, the patient reported a "helminth therapy" as complementary treatment for the autoimmune disease (2.500 *Trichuris suis* ova and 30 *Hymenolepis diminuta* cysticercoids every three weeks during the preceding four months). The last *T. suis* intake was 4 weeks, the last *H. diminuta* intake 6 to 8 weeks ago. Infection with hookworms was not reported.
On admission the physical examination was unremarkable. During the workup a colonoscopy revealed the presence of many white-yellowish, round worms fixed to the mucosa surface showing slow movements. Biopsies from the colon yielded eosinophilic infiltrates consistent with parasitic infection. Analysis of one complete and two fragmented worms showed at least one mature female trichurid identified by typical eggs emerging from the uterus. Stool examination revealed Trichuris and hookworm eggs at a concentration of 27 and 39 eggs/g feces, respectively. Travelling outside of Europe was denied.
Conclusion: The findings in this case are consistent with the reported iatrogenic infection. The detection of Trichuris eggs together with an egg-laying worm 4 weeks after the last egg intake indicate a persistent whipworm infection which, to our knowledge, had not yet been demonstrated after worm therapy. Persistent infection might probably be due to concomitant immunomodulatory therapy with hydroxychloroquine.

Phylogeny and evolution

PAE-P-01

Molecular Identification and Characterization of *Theileria* spp responsible for Ovine Theileriosis in Egyptian Oases

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Abstract
Introduction: Tick-borne hemoprotozoan parasites like *Theileri*a spp is among the most economically important infections of small ruminants all over the world especially in tropical and subtropical regions. *T. lestoquardi and T. ovis* are the main cause of ovine theileriosis **(Guo et al., 2002; El Imam et al., 2015).** In Egypt there is no enough data about the molecular characterization of these species especially in Upper Egypt and Egyptian Oases.
Objective: This study was conducted for molecular identification and characterization of *Theileria* spp. of sheep in the Egyptian Oases.
Materials and Methods: Study was conducted on 59 sheep blood samples collected from three different localities in New-Valley governorates (Egyptian oases), Egypt. The extracted DNA from sheep blood were subjected to PCR using primers targeting 18S ribosomal RNA gene; followed by sequencing and phylogenic analysis **(AL-Hosary et al., 2015; Elsify, et al., 2015).**
Result: Out of 59 samples only four samples were positive by PCR for 18s rRNA gene. The nucleotide blast of the generated sequences revealed that three sequences were *theileria ovis* ((KY494648, KY494649 and KY494650) while only one was *theileria lestoquardi* (KY494651).

Conclusion: This paper considers the first molecular report of *theileria ovis and theileria lestoquardi* in sheep in Egypt accompanied with phylogenic analysis. This infection is one of the destructive obstacles for sheep production in the Egyptian Oases and need more investigation in the future to evaluate its epidemiological situation and construct plans for eradication.
Key Words: Sheep, *Theileria ovis, Theileria lestoquardi*, PCR.

Figure 1

Figure 2

PAE-P-03
Diversity of *Eimeria* spp. in different mouse genotypes across the European Hybrid Zone

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Eimeria is the most diverse genus in the phlyum Apicomplexa and different species infect several domestic animals and wildlife. Nevertheless, evolution and diversification of host-parasite interactions remains barely investigated using closely related host species. For that reason, we study *Eimeria* spp. in the European house mouse hybrid zone.
The aim of this study is to describe the phylogenetic diversity of *Eimeria* and to test for potential preference or specificity for the two host subspecies. Using a multi-marker and multi-genome approach we relate phylogenetics with morphometrical characteristics and tissue preference of the

parasites. Colon content and feces from mice collected in Germany and Czech Republic were used to amplified sequences of 3 markers (18S, COI and ORF470). Sequences from 85 infected mice were used for phylogenetic and haplotype analysis. Morphometrical analysis was conducted with sporulated oocysts from the different phylogenetic groups and tissue preference was assessed using qPCR on DNA from cecum and ileum tissue.
According to the phylogenetic and haplotype analysis, three major clades or haplogroups of *Eimeria* were defined (A, B and C). Morphometrics and tissue preference for these haplogroups are in agreement with descriptions for species with similar or identical sequences in databases: E. falciformis (A, caecum), *E. ferrisi* (B, caecum) and *E. vermiformis* (C, ileum). Concerning host specificity, *E. falciformis* shows a preference for the western house mouse, *E. vermiformis* for the eastern house mouse, and *E. ferrisi* is a generalist.
We were able to distinguish three species of *Eimeria* infecting two closely related subspecies of the house mouse across a large geographical area. Our ability to differentiate these parasites and their host preferences lays a foundation for work on host-parasite interactions in the natural laboratory of the house mouse hybrid zone.

PAE-P-04
Ecology of Corynosoma infection in Sea Otters and Seals as an example of parasite diversity and evolutionary divergence.

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According to a 2003 study by Mayer, Acanthocephalan infection affected 94.4% (Corynosoma enhydri) of southern sea otters (Enhyrda lutris nereis). While the study claims Corynosoma causes low mortality and therefore no significance, the physiological impact of such a high frequency of non-lethal infection has not been investigated. Today little is known about the Corynosoma species infecting sea otters. While C. enhydri was identified over 100 years ago (Luhe 1904), it"s intermediate or paratenic host have not been identified. There is also speculation that Corynosoma found in the southern sea otter may not be the same species as the Alaskan population, as it is significantly smaller than those found in the northern otter and a hundred year bottleneck due to otters near extinction (Mayer 2003).
Seals predate otters in their evolution, and Corynosoma in sea otters most likely evolved from seals due to their close genetic relation (Garcia-Varela et al. 2005). This proposed project aims to explore the evolutionary history of Corynosoma in otters, focusing on the ecology of acanths in sea and river otters, as it relates to the genetic history of Corynosoma in otters and seals.
Objective 1: Identify Corynosoma sp. in the Alaskan and Southern Sea Otter; using gene sequencing to determine the genetic similarities or differences between the populations in Alaska and California. Compare against C. strumosum found in seals and river otters along the coasts of Germany.
Objective 2: Compare Parasite prevalence and intensity/severity of infection in the intestinal tracts of sea otters, relate to similar infection in river otters and harbor seals in Germany. Focusing on species composition, severity, and distribution in the GI tract.
Objective 3: Compare ecological factors possibly affecting the distribution, prevalence, or host selection for Corynosoma. Age, location, diet, and pollutants – relate to life history/transmission pathways of parasite, immunology etc.

PAE-P-05

Phylogenetic analysis of UDP-glycosyltransferase family from the parasitic nematode *Haemonchus contortus*

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Introduction: The UDP-glycosyltransferases (UGTs) represent a major group of phase two drug-metabolizing enzymes, playing an important role in the detoxification of xenobiotics, including many drugs. UGTs were recently identified as possible players in the resistance mechanisms.
Objectives: This study explores the genetic characterization of the UGT family from sheep gastrointestinal parasite *Haemonchus contortus*, including its comparison with UGTs from free living nematode *Caenorhabditis elegans*.
Material and Methods: Draft *contortus* genome was explored, predicted amino acid sequences of 37 *H. contortus* UGTs and 60 *C. elegans* UGTs were aligned and consensus Neigbor-joining phylogenetic tree was constructed using MEGA7.
Results: The comparative analysis revealed that homologues for single-member UGTs in *elegans* are present in *H. contortus* and the majority are single-copy genes in the parasite as well. *H. contortus* seems to lack the dramatic gene expansions seen in some *C. elegans* UGT families; only two less expanded homologous clusters in the parasite (UGT366 and UGT365; 6 and 7 genes, respectively) seem to align well. Several of the UGT genes are very similar and are present in clusters within a short proximity in the genome; hence appear to have arisen through repeated gene duplications.
Conclusion: The UGT diversity and local gene expansions might reflect adaptation to toxin and pathogen exposure. The future research of gene expression levels in various *contortus* isolates and exploring selected UGTs activities can reveal potential contribution to resistance mechanisms in this parasite.
This project was supported by Czech Science Foundation (Grant No. 17-11954Y) and by Charles University (PRIMUS/17/SCI/4).

PAE-P-06

Survey of Haemosporidian Parasites in Bats in Ngounié Province, Gabon

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Human malaria is caused by single-celled intracellular eukaryotic parasites that belong to the haemosporidian genus Plasmodium (Haemosporida). Understanding the evolution of malaria parasites

and their phylogenetic context is key to understanding the important human disease. Parasites of the genera Nycteria and Polychromophilus are close relatives of Plasmodium, but lack the erythrocytic schizogony. Nycteria parasite replication occurs exclusively during liver infection, whereas Polychromophilus features schizonts in the lumen of pulmonary vessels and also in the liver, kidneys and adrenals. Blood stages in both genera are limited to sexual stages. Nycteria and Polychromophilus parasites exclusively infect bats. Here, we present data from investigations of haemosporidian infections in bats in the province Ngounié, Gabon, in 2015. A total of 57 samples of four species of fruit bats and insectivorous bats of five different genera were obtained during the capture release study. Small blood samples were collected as thin blood smears and preserved on DNA FTA cards. Microscopy and molecular methods were used to detect and identify the haemosporidian parasites and to investigate their phylogenetic relationships among the large group of malaria parasites. The study detected Nycteria infections in five individuals of Rhinolophus as well as Polychromophilus in two individuals of Miniopterus bats. Interestingly, no parasites of the genus Hepatocystis were found in the investigated epauletted fruit bats, although high prevalences of this parasite genus are usually common in these bats. Phylogenetically, the sequences of the two detected Polychromophilus parasites group closely with Polychromophilus melanipherus, a species that is common in Miniopterus bats in Europe and a Polychromophilus sequence from a Miniopterus host from Guinea, pointing to a host genus specific subclade of Polychromophilus. The Nycteria parasite sequences of the current study group within a clade of Nycteria parasites from other African Rhinolophus bats and might represent the species Nycteria congolensis, which indicates that this parasite species is common and widespread in African rhinolophids. Together, our results add important molecular information to two neglected haemosporidian genera.

Physics of parasitism

POP-P-01

From solitary swimmers to swarms and back – trypanosomes on their journey through the tsetse fly

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Trypanosoma brucei undergoes a complex life cycle in the tsetse fly vector. The parasite´s development occurs during a journey through the different microenvironments of the fly´s internal organs. For the trypanosomes this involves crossing various barriers, confined surroundings, and swimming against flow and peristaltic movement. Additionally, they undergo major and drastic morphological changes. The parasite´s motility, which is directly dependent on morphology, is essential for its survival and successful development.
This work details cell morphology, motility, and collective behaviour of different developmental stages from the tsetse fly using high spatiotemporal resolution microscopy. Using fluorescently labelled parasites, swimming patterns of solitary swimmers were analysed *in vivo* and *in vitro,* as well as collective motion at the single cell level *in vivo*. We show that trypanosomes are able to synchronise their flagellar beats and produce superordinate wave patterns at high cell concentrations, probably by hydrodynamic self-organisation inside the fly interstices.

Additionally, light sheet fluorescence microscopy was established as a powerful tool for the 3D-analysis of the infection process in the tsetse fly´s digestive tract. The results provide information about tissue geometry and topology with unprecedented resolution. We were able to visualise fluorescent trypanosomes inside the surprisingly complex folds of the peritrophic matrix at a single cell level. Calculations using 3D Euclidean distance mapping allows us to measure the void space for trypanosomes to navigate within this labyrinth.
In summary, we provide a detailed view on trypanosome motile behaviour as a function of development in diverse host surroundings. We propose that the infection process is an alternating succession of solitary and collective motions, adapted to the varying and convoluted microenvironments inside the vector.

POP-P-02
Structural basis for the protective function of the dynamic variant surface glycoprotein coat of African trypanosomes

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The bloodstream form of *Trypanosoma brucei* protects itself from attack by the host"s immune system by covering its surface with a densely packed yet highly mobile coat, composed nearly exclusively of a GPI-anchored variant surface glycoprotein (VSG). Despite the importance of this coat for parasite survival, surprisingly little structural information is available for the family of VSGs. In particular, addressing the structure of the complete VSG has proven difficult. Therefore, the structure-function relationship for this protein still remains largely unknown.
We employed small angle x-ray scattering (SAXS) in combination with the high-resolution crystal structures of the N-terminal domain and NMR-structures of the C-terminal domain (CTD) of the two VSGs MITat1.1 and ILTat1.24 to solve the first complete VSG structures. Both VSGs show great flexibility between domains, suggesting that VSGs can adopt two main conformations. This would allow for adaptive responses to obstacles or changes in protein density, while maintaining a protective barrier at all times. Although the CTD-types of VSGs MITat1.1 and ILTat1.24 influence the overall membrane occupancy of the proteins, relaxed and compact conformations exist for both VSGs. Our findings emphasise the importance of the structural flexibility of the VSG in maintaining a functional trypanosome surface coat. The structural findings are supported by the detection of two freely diffusing populations of VSG protein by single molecule diffusion measurements in supported lipid bilayers.

Prevention

PRE-P-01

Routine surface disinfection when working with free-living amoebae (*Acanthamoeba* spp., *Balamuthia mandrillaris*) trophozoites and cysts

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Introduction: Experimental research in free-living "opportunistic" amoebae is broadly motivated. All *Acanthamoeba* spp. are listed biosafety level-2, *B. mandrillaris* and *Naegleria fowleri* BSL3. Cysts are resistant to chemical and physical conditions adverse to other eukaryotes. Formaldehyde is now considered a carcinogen (Category cB). We were obliged to look for an alternative routine surface disinfectant that is least harmful to human health, as well as both trophozoiticidal and cysticidal.
Objectives: To develop test systems relevant to daily practice, to select chemical disinfectants efficacious against trophozoites and cysts in a short exposure time, with the best human and environmental tolerability and longest shelf life, to verify these results with different FLA species.
Materials & Methods: *A. castellanii* Neff, *A. castellanii* 1BU and *A. culbertsoni* cysts were induced in Hirukawa medium, then positively selected with SDS. Surface decontamination was tested by coating stainless steel plates with 1-5x105 amoebae and wiping once or twice with a tissue soaked with disinfectant. After 5 min plates were sampled with a cotton swab that was then twirled in culture medium. For decontamination in suspension, amoebae were mixed with disinfectants. After 3 min these were neutralized by extensive diluting and 2x washing by centrifugation. 14 d cultures revealing any viable trophozoites were rated "positive".
Results: Alcohol-based disinfectants were less effective against trophozoites and quasi ineffective against cysts. Formaldehyde-, hypochlorite-, organic peroxides-, quaternary ammonium-based disinfectants were all effective against trophozoites, but only hypochlorite and quaternary ammonium was fully cysticidal at test conditions.
Conclusions: Alcohol-based hand disinfectants cannot be recommended. Handling, human tolerability and shelf-life favor quaternary ammonium-based disinfectants for routine surface disinfection over hypochlorites and formaldehydes.

Protozoan parasites

PRO-P-01

Multifunctional Single Domain Antibodies For Targeting Protozoan Parasites

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Protozoan parasites such as *Plasmodium, Leishmania* and *Toxoplasma gondii* are responsible for some of the most severe health problems worldwide. The intracellular forms of protozoan parasites express on their cell surface a dense cover of glycosylphosphatidylinositol (GPI) lipids. In contrast to the

protein bound human GPI lipids, vast majority of GPI lipids from protozoan parasites are expressed as free-forms or attached to extracellular glycoconjugates (1-2).
In Malaria, *Plasmodium falciparum* derived free-GPI lipids are believed to play important role as toxins and were shown to modulate the host's immunopathological responses during infection (3). Although sharing a conserved core structure with human GPI lipids, the different types of core side chains make free-GPIs from protozoan parasites an attractive anti-parasitic targets.
Single domain antibodies, (sdAbs, also termed "Nanobodies" or "VHH" (variable domain of heavy chain)), are unique subclass of IgG antibodies that are produced mainly in Cameloids. Unlike conventional IgGs, sdAbs are small antigen binding molecules (15kD), easily manipulated genetically and readily expressed both recombinantly or intracellularly. Using native and synthetic *Plasmodium falciparum* GPIs, we aim to develop a multifunctional single domain antibody, able to specifically target GPI lipids from multiple protozoan parasites.

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PRO-P-02

***Babesia* spp. in wild cervids species**

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Introduction: Babesiosis is an emerging zoonotic disease and various wildlife species are reservoir hosts for zoonotic species of *Babesia*. In Europe, *B. divergens*, *B.* sp. EU1 proposed as *B. venatorum* are capable of causing severe disease in man, and most cases are from spleenectomised patients. *Babesia* sp. EU1 has also been identified in roe deer, and *B. divergens* and *B. capreoli* have been described as occurring in wild European cervids.
Objectives: The objective of the present study was to investigate the presence and prevalence of *Babesia* spp. in wild cervids.
Materials & Methods: From 2013 to 2016, spleen samples were collected from 203 free ranging ungulates (99 moose, 67 roe deer, 37 red deer) in the southern part of Norway. The DNA was amplified by PCR using two different PCR protocols conventional PCR and RT-PCR for *Babesia* spp. 18S RNA. PCR products after conventional PCR amplification were analysed by 1.5% agarose gel. Amplicons obtained from amplification were purified, obtained sequences were compared with sequences available from the GenBank database. Phylogenetic analyses were conducted using MEGA6 software.
Results: DNA of *Babesia* spp. was found in all tested cervids species. By using conventional PCR, 4 out of 99 moose (4 %), 35 out of 67 roe deer (52.2%), 12 out of 37 red deer (32.4 %) were positive for *Babesia* spp. The sequence data revealed that *Babesia* spp. sequences of wild cervids species were similar with *B. capreoli*, *B. divergens*, *B. venatorum, B.* cf. *odocoilei, B. odocoilei.*
Conclusion: We detected a high prevalence of *Babesia* spp. DNA in roe deer (52.2 %) and red deer (32.4%) and low prevalence in moose (4%).

PRO-P-03

Development and application of a recombinant protein based indirect ELISA for the detection of serum antibodies against *Cystoisospora suis* in swine

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Introduction: Cystoisosporosis, caused by an apicomplexan parasite *Cystoisospora suis,* is one of the predominant pathogens in suckling piglets with a global prevalence of over 70%. Currently, for experimental studies, indirect fluorescent antibody test (IFAT) is the only available serological tool for detecting antibodies against *C. suis* despite several limitations, including relatively subjective interpretation and low throughput. In the present study, an indirect enzyme-linked immunosorbent assay (ELISA) was developed using a recombinant merozoite protein for the detection of serum antibodies against *C. suis* infection.
Materials and methods: rCSUI_005805 was expressed in *E. coli* as N-terminal HIS fusion protein, specificity of which was confirmed in an immunoblot probed with *C. suis* positive sera. Optimal dilutions of recombinant protein, sera and conjugate were determined by checkerboard titrations. Agreement between IFAT and newly developed ELISA was accessed with kappa statistics. The receiver operating characteristic (ROC) curve analysis based on 185 serum samples with known *C. suis* exposure tested in the reference IFAT was used to determine the cut-off value, sensitivity and specificity.
Results: The optimal cut-off based on ROC analysis was a 1:800 diluted serum sample OD value of 0.82 for which the sensitivity and specificity values were 94.7% (95% CI 89.5 - 97.8%) and 98% (95% CI 89.5 - 99.5%), respectively. This was comparable to the cut-off value of 1.17, calculated as the mean optical density of 51 *C. suis* negative sera plus three standard deviations. According to kappa coefficient, an excellent correlation (κ>0.8) was found between IFAT and the established ELISA.
Conclusion: The diagnostic accuracy measured as the area under the ROC curve (AUC) index was 0.98, indicating excellent discriminatory capacity of the test and its possible application as a marker for infection or exposure in large-scale epidemiological studies.

PRO-P-04

Declining trend of malaria and high efficacy of Artemether-Lumefantrine(Coartem®) against P.falciparum in Ziway Dugda district, Ethiopia.

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Introduction: Malaria has been one of the major public health problems in Ethiopia for decades. As the country is planning to eliminate malaria by 2030, there is a need to assess the trend of malaria and efficacy of antimalaria drugs in those malaria endemic areas such as Ziway Dugda district.
Objective: The main aim of this study was to assess the temporal trend of malaria prevalence and to evaluate the efficacy of Artemether-Lumefantrine(AL) against *P.falciparum* in Ziway Dugda district.
Materials & Methods: Both community based cross sectional and institutional based interventional study designs were implemented to assess the prevalence of malaria and efficacy of AL. Eight years retrospective data of blood film examination was collected and blood films from randomly selected community members were examined. Patients with *P.falciparum* mono infection (parasite density of 1000–100,000 parasites/ ml) were treated with AL. Level of parasitemia were determined microscopically at base line and 3,7,14 days after the initiation of treatment.
Result: Microscopically confirmed malaria cases declined from 821 cases per year in 2010 to 72 cases in 2017. From 35,276 malaria suspected patients tested in the district's health center, 5,368(15.2%) were malaria positive microscopically, with 3,533(65.6%) *P.vivax ,* 1,819(33.8%) *P.falciparum,* and 16(0.3%) mixed infections. About half, 2489(46.3%), of the malaria infected patients were under five children. From 657 tested community members, 24 (3.6%) were infected with malaria parasite. Fifteen (62.5%) of them were infected with *P.vivax,* 6(25%) with *P.falciparum* and 3(12.5%) had mixed infection. All (107) patients treated with AL were able to clear the asexual stage of the parasite at day three.
Conclusion: Trend of malaria is declining with shift in dominancy of the circulating species *(P.vivax*). AL was found to have high efficacy against P.*falciparum* in Ziway Dugda district.
Key words: Malaria, Trend, Artemether-Lumefantrine, Resistance, Ethiopia

Figure 1

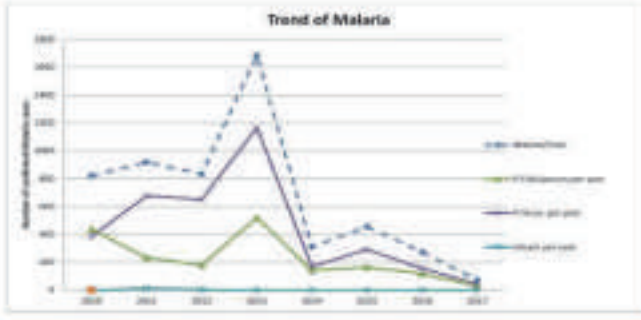

Figure 2

Table 1. Multivariate logistic regression analysis of malaria prevalence and associated factors among inhabitants of Zi-way Dugda woreda, Ethiopia

Variable	Malaria		AOR	95% CI	P value
	Negative (N(%))	Positive (N(%))			
Age of study participants					
Under Five					
Above or equal to five					
House was sprayed with IRS					
Yes					
No					
Presence of stagnant water near by the household					
Yes					
No					

Note: Twenty four of the microscopically confirmed Malaria cases were found in 22 households.

PRO-P-05
Cell penetrating peptides (cpp) a delivery tool for anti-cryptosporidium drugs

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Cryptosporidium are small apicomplexan intracellular parasites that are located in the gut adjacent to the fecal stream and infects many various hosts including human, cattle, goats and pigs (Olson et al., 2004). In general, a *Cryptosporidium* infection in healthy humans is characterized by heavy but self-curing diarrhea, nausea, vomiting, and abdominal pain. In contrast, infection of immune compromised subjects or small children, especially when they are malnourished, can become life threatening if not efficiently treated (Ventura et al. 1997; Kotloff et al., 2013). The introduction of highly active anti-retroviral therapy against HIV infection has significantly reduced the burden of *Cryptosporidium*

infection in industrialized countries. However, in developing countries cryptosporidiosis is a major cause of diarrhea in children, especially in those under 2 years of age where the pathogen is the second most frequent cause of moderate to severe diarrhea just behind rotavirus infection, and heavily associated with mortality. For most drug candidates the transition from *in vitro* experimental data to *in vivo* application often represents an insuperable challenge due to poor record of systemic bioavailability and toxicity. Removing this hurdle would greatly facilitate the transition of *in vitro* studies to *in vivo* application in the process of drug development. Drug combination to cell-penetrating peptides such as octa-arginine, represent an attractive possibility for the targeting *Cryptosporidium* because they can greatly enhances the uptake of the drugs into the intracellular parasite (Sparr et al. 2013). The goal of the current study aims at using the cell-penetrating peptide beta octa-arginine to enhance the uptake of anti-Cryptosporidium drugs thereby improving their efficacy.

PRO-P-06

Mutual influences of the apicomplexan parasites *Toxoplasma gondii* and *Eimeria tenella* in poultry macrophages

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Toxoplasma (T.) gondii and *Eimeria (E.)* tenella are two common parasites in poultry. Mixed infections are likely to occur frequently in chickens due to the high prevalence of both pathogens. In this study, we investigate the co-occurrence of the two pathogens in the same immune-competent host cell population towards potential parasite-parasite as well as altered patterns of parasite-host interactions. Primary macrophages from chicken blood were co-infected *in vitro* with *T. gondii* tachyzoites (RH strain) and *E. tenella* sporozoites (Houghton) for 72h. Morphologic observations by light microscopy and assessments of parasite replication by quantitative real-time PCR (qPCR) were performed at 24, 48, 72h post infection. Six immune factors which could be altered by *T. gondii* or *E. tenella* infection are selected in this study. Higher morphologic changes of macrophages were distinct while mixed infection at 24h p. i. while immunologic activation was mainly seen. mRNA expression of iNOS, IL-10 and TNF-α by macrophage showed significantly higher in mixed infection in different time point. At 72h p. i. the total number of macrophages decreased distinctly as well as the number of replicates for both parasites. By qPCR, *E. tenella* population was less suppressed during *T. gondii* co-infection while *T. gondii* replication was not hampered. First investigations in this *in vitro* infection model show obvious interaction of concurrent infections with *T. gondii* and *E. tenella* and will help to understand the importance of natural co-infections of chickens and risk of infection for human by poultry meat.

PRO-P-07
The production and characterization of novel β-carbonic anhydrases of *Trichomonas vaginalis*

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Introduction and Objective: The widespread use of antibiotics has had a profound impact on global health. Anti-infective-resistant strains of pathogens limit current treatment options, and have created a demand for alternative medication. Among promising novel drug targets are carbonic anhydrases (CAs). They are ubiquitous enzymes responsible of catalyzing the reverse hydration of carbon dioxide into bicarbonate ions and protons. CAs are encoded by seven evolutionarily distinct gene families: α, β, γ, δ, ζ, η and θ. The human genome contains only α-CA genes, while many clinically significant pathogens express only β-CAs. This makes β-CAs potential targets for therapy against many clinically significant bacterial and parasitic infections. The production and characterization of β-CAs of pathogenic organisms enables design of effective inhibitors that block their enzymatic function. Ideally, drugs developed on this basis would ultimately lead to elimination of target pathogens without affecting human α-CAs. *Trichomonas vaginalis* is a protozoan parasite that causes one of the world"s most common STIs, trichomoniasis. It has two β-CA isoforms (TvaCA). The objective of this study was to produce TvaCAs as recombinant proteins, and to determine their structure, and kinetic and inhibition profiles.
Materials and Methods: TvaCAs were expressed recombinantly, and purified with affinity chromatography. The proteins were confirmed by SDS-PAGE, Western blotting and proteomics. Protein structure was determined by X-ray crystallography.
Results and Conclusions: The X-ray crystallography confirmed the TvaCA1 as a dimer. Second TvaCA is still undergoing structural analysis. The kinetic studies showed that both TvaCAs had significant catalytic properties for the hydration of CO2 to bicarbonate and protons, with the following kinetic parameters: kcat of 4,9 x 105 s-1 and a kcat/KM of 8.0 x 107 M-1 s-1 (TvaCA1), and kcat of 3.8 x 105 s-1 and a kcat/KM of 4.4 x 107 M-1 s-1 (TvaCA2). The enzyme activity was inhibited in nanomolar range by sulfonamides and some other small molecules such as boronic and phosphonic acids. Further work to detect more potent TvaCA inhibitors is warranted.

PRO-P-08
***Sarcocystis nesbitti* in Australia? New discovery raises question about lifecycle**

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Sarcocystis nesbitti is so far the only *Sarcocystis* species known to infect humans as intermediate hosts. The supposed lifecycle involves nonhuman primates as intermediate and snakes as final hosts.

One objective of this study was to identify the final host. For this purpose, faecal samples were collected from 75 reticulated pythons (*Malayophyton reticulatus*), 5 green tree pythons (*Morelia viridis*) and 3 Burmese pythons (*Python bivittatus*) from Southeast Asia and 23 scrub pythons (*Simalia amethistina*), 6 spotted pythons (*Antaresia maculosa*) and 3 black-headed pythons (*Aspidites melanocephalus*) from Northern Australia. Presence of sporocysts was determined microscopically and 18 randomly picked, sporocyst-positive samples (11 from SE Asia, 7 from Australia) were further processed by molecular methods. PCR and sequencing of the 18S rDNA were performed with single sporocysts. Phylogenetic analysis with the obtained sequences and those of already published Apicomplexan species was carried out.
Sarcocystis spp. prevalence was generally high across the study area (68%). Among the 18 randomly picked positive samples, the only *Sarcocystis* species detected from SE Asian was *S. singaporensis* (in reticulated pythons), which was absent from all Australian samples. We distinguished three different *Sarcocystis* spp. in the Australian sample set; two were excreted by scrub pythons and one by the spotted python. The sequence of the latter is an undescribed *Sarcocystis* species. Of the two *Sarcocystis* species found in scrub pythons, one showed an 18S rRNA gene sequence similar to *S. zamani*, the second sequence was homologous to *S. nesbitti.* The presence of *S. nesbitti* in Australia raises the question about the intermediate host and the previously assumed lifecycle of this parasite in general, since nonhuman primates are not present on the Australian continent. Further studies are needed to identify the main hosts involved in the lifecycle of this unique human pathogenic *Sarcocystis* species.

PRO-P-09

Synthesis and *in vitro* characterisation of novel inhibitors of *Trypanosoma cruzi* histone deacetylase 2 (tcDAC2)

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The American trypanosomiasis, also known as Chagas disease, has been recognized by the WHO as one of the neglected tropical diseases and is estimated to affect 8 million people worldwide.[1] This disease is caused by the protozoan parasite *Trypanosoma cruzi,* which is transmitted by triatomine bugs. Endemic in Latin America, Chagas disease has spread from its original boundaries through migration and so becoming a global issue.[2] Current treatment is limited to benznidazole and nifurtimox, which are associated with severe side effects, low cure rates in the chronic stage and the emergence of drug resistance.[3] Histone deacetylases (HDACs) are validated drug targets in cancer therapy and there is evidence that they can also be adressed to treat parasitic infections. In *T.cruzi* four zinc dependant HDACs has been identified. We focus on the *Trypanosoma cruzi* deacetylase 2 (TcDAC2), the homologous isoform of smHDAC8 in *Schistosoma mansoni*, which was identified as a

promising target for antiparasitic drug discovery.[4] Recently, we tested several in house HDAC inhibitors on tcDAC2 *in vitro*. Aromatic hydroxamic acids were identified as promising hits that are able to kill the parasite in an i*n vitro* assay. The synthesis and biological characterisation of novel tcDAC2 inhibitor is being carried out.

This work is funded by the European Union's Seventh Framework Program for research, technological development and demonstration under grant agreement no. 602080 and by the European Regional Development Fund of the European Commission (K.H. and W.S.).

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PRO-P-10
Phylogeography of dsRNA viruses of *Leptomonas pyrrhocoris* (Trypanosomatidae, Kinetoplastea)

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Viruses play an important role in regulation of gene expression and general post-transcriptional processing of RNA in eukaryotic cells. Viruses, and double-stranded RNA (dsRNA) viruses in particular, can be found in any cellular life, explaining their immense diversity. The molecular characterization of the dsRNA viruses from *Leishmania* and their association with pathogenesis of the disease and survival of the parasites, engendered a new field focused on dynamic relationships between protists and viruses.
In this work, we analyzed numerous cultures of the monoxenous trypanosomatid *Leptomonas pyrrhocoris*, a parasite of the firebug *Pyrrhocoris apterus*, for the presence dsRNA viruses. Firebugs were collected in different localities around Europe (Czech Republic, France, Germany, Lithuania, Poland, Romania, Russia, Slovakia, and Ukraine) and their flagellate parasites were examined for the presence of previously described tombus-like viruses, ostraviruses, and other dsRNA viruses. The total RNA was extracted from log phase-grown cultures, treated with DNase I and S1 nuclease and visualized by gel electrophoresis. In addition to two previously documented viruses, we have revealed the presence of several novel RNA viruses in this species, confirming our previous notion that it is, indeed, a "hotbed" for viral discovery.
Future work is warranted to further characterize these new viruses and establish the roles they might play in ecology, physiology, pathogenesis and evolution of the monoxenous *Leptomonas pyrrhocoris*.

PRO-P-11
Control of the expression of the variant surface glycoprotein of African trypansomes

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African trypanosomes, including *T. brucei* are strictly extracellular parasites of medical and veterinary importance. The parasites thrive in the host despite a strong immune challenge due to a unique antigenic variation of the variant surface glycoproteins (VSGs). Switching of the VSGs may occur as a result of DNA rearrangement (gene conversion and telomere exchange) or transcriptional regulation (in-situ switch). The molecular processes as well as the order of events during a switch are however unclear. Recent evidences have shown that during an in situ switch transcription of a new expression site (ES) is activated before silencing/attenuation of the active ES. Conserved motifs (16mer and 8mer) in the VSG 3'UTR have been identified as potential regulatory elements in this process. The 16mer motif within the VSG 3'UTR has been shown to play a role in stabilization of VSG mRNA. Scrambling of this motif also leads to switching in stable double expressers depending on the genomic location of the defective 16mer. The motif therefore appears to mark the functionality of the ES. This study further investigates the role of the 16mer motif in VSG expression.

PRO-P-12
Profiling of membrane transport protein expression in the *Plasmodium berghei* life cycle exemplified by CRT and ATP4

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Membrane transport proteins (MTPs) transport nutrients, metabolic products and inorganic ions across membranes. Accordingly, many MTPs are essential for *Plasmodium* life cycle progression, including maintenance of cell physiology and adaptations to environmental changes. Furthermore, MTPs play a major role in malaria therapy, since pharmacologically active compounds can be transported away from the site of action. For instance, mutations in the Chloroquine Resistant Transporter (CRT) lead to a reduction of chloroquine in the digestive vacuole and confer chloroquine-resistance to the parasite [1]. One MTP, the cation-ATPase ATP4, is a promising drug target, since it is inhibited by the spiroindolone SJ733, potent inhibitor of *P. falciparum* blood stage replication [2]. In both cases, the endogenous functions and their cargo remain enigmatic. An important step towards a functional classification is the determination of the expression during life cycle progression and their intra-cellular localization.
Here, we present a quantification of the spatio-temporal expression of *P. berghei* CRT and ATP4 during the entire life cycle in the insect vector and mammalian host. Live fluorescence microscopy of transgenic *P. berghei*-parasite lines that harbour endogenously tagged MTP::mCherry and digital image processing *via* ImageJ permits a systematic analysis in comparison to normal wildtype parasites

Our study shows expression of CRT and ATP4 beyond asexual blood stage parasites, suggestive of additional functions in other life cycle stages.

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PRO-P-13
Innovative octaarginine-based tools to boost *Cryptosporidium* research

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In general, efforts to develop anti-*Cryptosporidium* drugs and vaccines have been hampered by the scarcity of adequate tools to investigate both the life cycle of the parasite and the mechanism of pathology *in vitro*. Most strikingly, the lack of an *in vitro* model that supports full life cycle of the parasite and the lack of appropriate reverse genetics tools to generate transgenic parasites are the essential hurdles in *Cryptosporidium* research. It has therefore been virtually impossible to design an appropriate platform to identify novel drug targets. Here we describe an innovative fast and efficient method based on the use of the cell-penetrating peptide oligoarginine to enhance the generation of transgenic Cryptosporidium strains. We also describe the use of oligoarginine to enhance anti-*Cryptospordium* Drug uptake and improve their efficacy.

PRO-P-14
Generation and characterization of selected virulence factor knockout strains of C. parvum using CRISPR/CAS method

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Cryptosporidium parvum is an intracellular yet extra-cytoplasmatic residing parasite, that infects the intestinal mucosa of animals and humans. It causes acute, persistent and chronic diarrhea and can become a life-threatening illness for immunosuppressed persons and malnourished infants. Despite being known as an important pathogen for many decades, research on this protozoon has been challenging, due to the lack of molecular tools, as well as the lack of an appropriate in vitro system that enables a continuous culture of the parasite. Several putative virulence factors of C. parvum have been described in the past, but their final role for infection remains to be proven. In this project we will use the CRISPR/Cas-method to knock-out specific genes encoding for virulence factors especially genes involved in locomotion and adhesion. The generated transgenic strains will be characterized using the COLO-680N cell line-based in vitro sytem and in vivo using the IFN-γ receptor knockout mouse model of cryptosporidiosis.

PRO-P-15
Glucose transport in *Cryptosporidium parvum* infected intestinal enterocytes

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Cryptosporidium parvum is a common threat in animal husbandry especially for young calves. In case of infection the parasite is able to cause massive damages in parts of the brush-border membrane which is important for the uptake of nutrients including glucose. It is well known that the uptake of glucose can be regulated depending on several wants and conditions. Aim of this project was to examine whether the intestinal epithelium is able to adapt glucose transport mechanisms in the presence of *C. parvum* and secure the glucose uptake nevertheless.
Therefore a new *C. parvum* infection model was established in IPEC-J2-cells (jejunal porcine enterocytes). To confirm successful infection, hsp70 gene was quantified by Real-time PCR. For screening a possible influence on the regulation of glucose uptake, gene expression of the glucose transporters Na-coupled glucose transporter (SGLT) 1, glucose transporter (GLUT) 1 and GLUT 2 in infected and uninfected cells was examined and compared. Furthermore the protein expression of SGLT 1 was measured in infected and uninfected cells and the actual glucose uptake was measured by using radioactively labelled 14C-alpha-methyl-glucose (AMG). Here we want to present a new infection model, which enables the illustration of interventions in the regulation of glucose transport caused by parasite infection. First results indicate an adaption of the glucose uptake, but further investigations have to be undertaken.

PRO-P-16
In vitro investigation of three oocyst specific proteins and their role for survival of Toxoplasma gondii oocysts in the environment

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Introduction: The genome of *Toxoplasma gondii* contains three small oocyst specific proteins annotated as so called "**l**ate **e**mbryogenesis **a**bundant domain-containing proteins" (Tg**LEA**). These proteins are thought to classify as "**i**ntrinsically **d**isordered **p**roteins" (**IDP**). It is assumed that such proteins don"t have a defined tertiary structure under normal physiological conditions. However, under specific stress conditions like desiccation or low temperatures they can adopt ordered structures, thereby protecting organelles or proteins from these stresses. It is also known that in oocysts trehalose synthesis is upregulated. This sugar is thought to provide protection against damage from desiccation in several animals and plants.
Objective: Our overall objective is to explore the role of TgLEAs for oocyst survival in the environment. More specifically, we want to investigate whether in surrogate hosts these proteins can aid in protection from desiccation and low temperatures, conditions that oocysts are also faced with in the environment.

Methods: Recombinant TgLEA-proteins are expressed in *Escherichia* coli and their biochemical properties reminiscent for IDPs are analysed. The *in vivo* impact of these proteins on survival of *E. coli* (wild-type as well as knock-out strain in the trehalose-synthesis pathway) after desiccation or incubation at low temperatures for extended periods of time will be analysed. Similar experiments will be conducted in the yeast *Saccharomyces cerevisiae* (wt and different knock-out strains known to be involved in stress defences) expressing the different TgLEA proteins. This will allow conclusions about their protective potential against environmental stress conditions.
Conclusion: Bioinformatic analyses show that all TgLEA protein sequences contain features of IDPs, like high overall hydrophilicity and enrichment in disorder-promoting amino acids. Expression of the TgLEA-proteins in *E. coli* resulted in soluble proteins which could be purified by affinity chromatography in high amounts. All three proteins showed aberrant sizes in gel filtration experiments, indicating non-globular (non-structured) folding, a hallmark of IDPs. The results of the growth assays under mentioned stress conditions will be presented.

PRO-P-17
Apicomplexan parasites, Toxoplasma gondii and Eimeria falciformis, induce and co-opt a master transcription factor c-Fos in the mammalian host cell

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Successful asexual reproduction of intracellular pathogens depends on their exclusive ability to exploit host resources and subvert antimicrobial defenses. Here we deployed two related parasites, namely *Toxoplasma gondii* and *Eimeria falciformis*, infecting the mouse to identify the host determinants of infection. Gene expression analyses of young adult mouse colon epithelial cells infected with either of these parasites demonstrated a remarkably distinct host-cell response, indicating that individual pathogens reprogram their intracellular niches in a notably tailored manner even though they are phylogenetically related and share the host cells (coccidians infecting mouse intestinal epithelium). As expected, we detected a series of host defense pathways, such as TLR, MAPK and mTOR signaling and cytokine-receptor interaction, majority of which are regulated by IFN-γ in response to infection. Moreover, pathways related to cell growth and differentiation are also regulated during infection phase. More importantly, our analyses revealed only two genes (encoding for c-Fos and Rab24 proteins) that were consistently induced by both parasites at early as well as late time points. Rab24 belongs to a small GTPase family possibly involved in autophagy, whereas c-Fos is a well-known master transcription factor (a part of AP1 complex) and a proto-oncogene, which governs a rich repertoire of cellular processes, including but not limited to, proliferation, apoptosis, inflammation and oncogenesis. Plaque assays using the c-Fos-/- mutated cells confirmed an apparently essential function of host c-Fos for the lytic cycle of *T. gondii*. In-depth phenotyping of *T. gondii* cultures in mouse embryonic fibroblasts the protein expression was not needed for efficient host-cell invasion or egression, but required for efficient intracellular replication. Accordingly, the asexual development of *E. falciformis* was significantly impaired in the c-Fos knockout cells. Taken together, our data signify potential subversion of a crucial host-cell regulator by the two intracellular parasites to promote their own development.

PRO-P-18

Comparison of the LightMix® Modular Assay Gastro Parasites with routine laboratory diagnosis for the detection of parasites in stool specimens from primary health care patients in Berlin, Germany

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Questions: Multiplex PCR assays might offer highly sensitive and specific laboratory tools for the detection of enteric parasites, which often depends on light microscopy and the performance of enzyme immunoassays (EIAs). The study aimed at comparing the LightMix® Modular Assay Gastro Parasites with routine laboratory procedures for the detection of enteric parasites.
Methods: Stool specimens submitted to the MVZ Labor 28 in June and July 2017 were consecutively examined by the LightMix® Modular Assay Gastro Parasites, which detects *G. duodenalis*, *E. histolytica*, *Cryptosporidium spp.*, *Blastocystis hominis,* and *Dientamoeba fragilis*, in comparison with EIAs detecting *G. duodenalis* or *E. histolytica/dispar* and light microscopy of wet mounts following enrichment.
Results: *D. fragilis* or *B. hominis* were detected in 131 (14.4%) and 179 (19.9%; 16 positive by microscopy; $P < 0.0001$) of 909 samples, respectively. Of 918 samples analyzed for *Cryptosporidium spp.*, six were positive by PCR and three of these could be confirmed by Kinyoun staining and another one by in-house PCR. *G. duodenalis* was detected by PCR, EIA, or microscopy in 20, 16, and 9 of 1039 stool samples, respectively; the four samples missed by EIA were confirmed by in-house PCR. In total, 938 stool samples were analyzed for *E. histolytica/dispar*, and nine of ten samples positive by EIA were negative by multiplex PCR. In-house PCR, however, identified *E. dispar* in all nine specimens. Notably, one *E. histolytica* infection (positive by both multiplex and in-house PCR) was missed by EIA and microscopy.
Conclusions: The LightMix® Modular Assay Gastro Parasites detected all parasites with high accuracy. Multiplex PCR assays for the detection of enteric protozoan parasites should be included in routine laboratory diagnostics.

PRO-P-19

***HSP101* overexpression aborts *Plasmodium berghei* pre-erythrocytic development**

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Host cell remodelling is critical for successful *Plasmodium* replication inside erythrocytes and achieved by targeted export of parasite-encoded proteins. In contrast, during liver infection the malarial parasite appears to avoid protein export, perhaps to limit exposure of parasite antigens by infected liver cells. We have previously shown that the ATPase of the protein translocon of exported proteins (PTEX), termed HSP101, is not expressed during early liver infection. We, therefore, hypothesised that this protein might be a limiting factor explaining the absence of PTEX-dependent export.
We generated transgenic *Plasmodium berghei* parasite lines expressing an extra copy of *HSP101* under the control of a range of strong and weak pre-erythrocytic and constitutively expressed promoters to

enhance parasite protein export in early liver stages. Parasites that express *HSP101* under a strong pre-erythrocytic promoter show severe growth defects in liver stage development *in vitro* and *vivo*. In good agreement, animals immunized with transgenic parasites display weak protection against sporozoite challenge infections. In contrast, parasites expressing *HSP101* under a weak constitutive promoter are able to complete the life cycle, but do not export PEXEL-proteins in early liver stages. Our results suggest that *HSP101* expression is tightly controlled to maintain sporozoite infectivity and PTEX-dependent early liver stage export cannot be restored by addition of HSP101, indicative of alternative export complexes or other functions of the PTEX components during liver infection.

PRO-P-20
Deciphering the molecular machinery of the antigenic variation regulator DOT1B

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Antigenic variation is an essential mechanism for survival of the protozoan parasite *Trypanosoma brucei* inside its mammalian host. This process is mediated by tightly controlled monoallelic expression of Variant Surface Glycoproteins (VSG) from one of 15 subtelomeric VSG expression sites (ES). At any given time only one single type of VSG is exposed on the surface of the parasite and periodically switching of this protein coat allows trypanosomes to evade the immune system of the vertebrate host. Switching of the coat can be either accompanied by transcriptional activation of a previously silent ES, a so-called *in situ* switch, or by homologous recombination of a different VSG gene from a large sub-telomeric reservoir into an active ES.
The conserved histone methyltransferase DOT1B exclusively trimethylates histone H3 on lysine 76 in trypanosomes and was shown to be involved in ES regulation. DOT1B-depleted cells show derepression of transcriptionally silent telomeric VSGs and extremely slow ES *in situ* switch kinetics. Furthermore, ES silencing during differentiation from the mammalian-infective stage to the insect-stage form is also slower compared to wild-type cells. Finally, the attenuation of the active ES in response to inducible expression of an additional VSG gene could not be observed in DOT1B-depleted parasites. The molecular machinery, which enables DOT1B to execute these different regulatory functions at the active ES is still elusive.
To better understand the mechanisms of ES regulation in *T. brucei*, we employed biochemical approaches to purify DOT1B-containing protein complexes. Using a combination of tandem affinity purification and proximity-dependent labeling of proteins with biotin (BioID), we identified several DOT1B-interacting proteins. Surprisingly, one of the most abundant DOT1B-associated protein complexes was RNase H2, which resolves DNA/RNA hybrids. A novel putative mechanism of ES regulation will be discussed.

PRO-P-21
Trans-species surface coats of African trypanosomes: What can they teach us about VSG functionality?

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African trypanosomes protect themselves from destruction by host defences by covering their surface with a dense coat of a single protein, the variant surface glycoprotein (VSG). The VSG and the coat it forms have been best characterized in Trypanosoma brucei. In this species the protein consists of a larger, elongated N- terminal domain that is exposed and a shorter, more secluded C- terminal domain consisting of one or two structured regions. Sequence variability in the N-terminal domain is fundamental for antigenic variation; how the C-terminal domain supports VSG functionality is however not clear. Other species such as T. congolense and T. vivax also possess a VSG surface coat, which presumably has the same protective function. Though similar, VSGs have evolved to differ from species to species, for instance T. congolense VSGs lack structured regions in their C-terminal domain and this is probably also the case for the T. vivax VSGs. In order to address the compositional differences in terms of functionality we attempted to generate transgenic T. brucei cells expressing VSGs of either of the other species on their cell surface. Whereas a T. congolense VSG could readily be expressed as the only VSG on T. brucei cells, this was not the case for the chosen T. vivax VSG. Supporting mutagenesis studies on T. brucei VSGs shed new light on the structure-function relationship in VSGs.

PRO-P-22
Identification of novel components of the histone methyltransferase DOT1A protein complex in *Trypanosoma brucei*

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DOT1 enzymes are evolutionary conserved histone lysine methyltransferases, which methylate histone H3 at lysine 79 (H3K79). This methylation mark is known to be associated with many cellular processes such as telomeric silencing, transcriptional control, DNA repair and development.
The unicellular parasite *Trypanosoma brucei,* the causative agent of the sleeping sickness, exhibits two DOT1 orthologs DOT1A and DOT1B. Both methylate the H3K79-equivalent H3K76. DOT1B, which tri-methylates H3K76, is involved in regulation of surface glycoproteins (VSGs) and stage differentiation of the parasite.
The second ortholog DOT1A mono- and di-methylates H3K76 in a cell cycle-specific manner and is (in contrast to DOT1B) essential for cell viability. Furthermore, we were able to demonstrate that DOT1A is responsible for replication regulation. RNAi-mediated knockdown of DOT1A abolishes replication completely. In contrast, overexpression of DOT1A leads to continuous re-initiation of replication within one S-Phase suggesting a H3K76 methylation-dependent replication initiation in *T. brucei*. However, the molecular mechanisms that regulate these processes are still elusive.

In order to learn more about replication control in trypanosomes, we used a tandem affinity purification-based method to identify DOT1A-interacting proteins. Surprisingly, we co-purified the ribonuclease (RNase) H2 complex, which was previously found to also interact with DOT1B. The RNase H2 heterotrimeric protein complex incises DNA at ribonucleotides, which were falsely incorporated during replication but is also important for genome integrity and embryonic development in mammals. Possible functions in context with DOT1 enzymes in trypanosomes will be discussed.

PRO-P-23

Giardia duodenalis in pets and their owners - a pilot study

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Introduction: *Giardia duodenalis* is one of the most important gastrointestinal parasites affecting humans and animals. A zoonotic potential is considered since human-pathogenic assemblages (A, B) were detected in the feces of different mammalian animals. Only few studies were conducted in Europe, which analyzed a potential zoonotic risk by testing fecal samples of animals and humans living in cohabitation.
Objectives: Aim of this monocentric prospective pilot-study was to assign assemblages of dogs and cats living in cohabitation with their owners in Berlin/Brandenburg, Germany.
Material and Methods: Analysis was performed with the ProSpecT Giardia EZ Microplate Assay (animal samples) and an immunofluorescence assay (MERIFLUOR, Cryptosporidium/Giardia, Meridian Bioscience; human samples). After DNA extraction, real time PCR (qPCR) and multi-locus sequence typing was performed at the triosephosphate isomerase- (tpi-), glutamate dehydrogenase- (gdh), beta-giardin-gene and ssurRNA.
Results: Fecal samples of 69 humans, 31 dogs and 7 cats were collected over 23 months. Thirteen (39%) canine, one (14%) feline and one (1.4%) human sample were tested *Giardia* positive with ELISA or immunofluorescence technique. Two additional human specimens were positive using qPCR. Assemblages could be assigned to six dog samples (2xA, 1x co-infection A/B, 1xC, 2xD) and two human samples but no cat-derived sample. One pair of samples (dog and human) had the same but not identical Assemblage B tpi sequence. The respective dog sample was also positive for assemblage A, which hampered sequence analysis.
Conclusion: The study highlights the need for better and standardized typing tools to distinguish *G. duodenalis* strains with higher resolution in order to perform proper case control studies for a realistic estimation of zoonotic risk.

PRO-P-24

The putative 2,4-dienoyl-CoA reductase of *Leishmania* represents a novel virulence factor

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Leishmania spp., are medically relevant protozoan parasites that belong to the trypanosomatids. This family contains monoflagellated parasites with monogenic or digenic life cycles. The genomes of several species have been sequenced and provide evidence for extensive lateral gene transfer events e.g. from bacteria. *Leishmania* spp. show a digenic life cycle and oscillate between life as extracellular promastigotes in the digestive tract of their insect vectors and an intracellular habitat in vertebrate hosts. A comparison of proteomes of amastigotes, purified from their intracellular habitat, to extracellular promastigotes showed that enzymes involved in ß-oxidation of unsaturated fatty acids, such as a putative 2,4-dienoyl-CoA reductase (DECR), become abundant in intracellular amastigotes. Phylogenetic analysis of *decr* revealed that this gene is present in a broad range of trypanosomatids, including genera *Leishmania*, *Trypanosoma* and *Angomonas* but seems to be absent in digenic parasites with an extracellular life style such as *T. brucei*. We have generated *decr*-deficient parasites. *In vitro* and *in vivo* infection experiments demonstrated that *decr*-deficient *L. major* lost virulence and cannot thrive inside host cells. Moreover, we monitored the degradation of 13C-labelled linoleic acid using GC/MS and LC/MS. Parasites deficient in DECR showed a considerable reduced amount of labelled citrate, an important intermediate in the TCA cycle generated from the final product of the β-oxidation, and an increased accumulation of polyunsaturated fatty acids compared to wild-type and complemented *decr*-deficient parasites. These findings are consistent with current views on metabolic adaptation to intracellular life mainly gained from studying *Leishmania*. In conclusion, proteomics analyses permitted the identification of a novel virulence factor in *Leishmania* that seems to reflect a key acquisition of an enzymatic property by a past event of lateral gene transfer linked to the evolution of intracellular survival capacity.

PRO-P-25
Literature overview on Apicomplexan infections in cheetahs *(Acinonyx jubatus)* and sympatric carnivore species

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Introduction: Little is known about parasite infections in free-ranging cheetahs. Cheetahs are listed as vulnerable in the Red List of the IUCN (International Union for Conservation of Nature), thus it is important to know with which pathogens and parasites cheetahs are infected and what the prevalence of these pathogens in the different cheetah populations is. There are many case reports of captive cheetahs, however, captive cheetahs are known to have a higher susceptibility to pathogens and diseases than free-ranging ones.
Objectives and Methods: This study presents an overview of the literature on parasite infections in free-ranging cheetahs and compares the results with the ones of captive cheetahs and sympatric carnivore species. Investigated parasites were *Toxoplasma gondii, Neospora caninum, Theileria-like Piroplasms, Hepatozoon sp. and Babesia sp.*
Results: Most studies were conducted in Namibia, in the Republic of South Africa and in Tanzania. Prevalence in free-ranging cheetahs varied from 0% to 100% depending on the parasite, with 1 to 40 investigated individuals. The study also identified study areas in which free-ranging cheetahs have not yet be tested for any of the above-mentioned parasites.
Conclusion: The large range of prevalence might depend on contact rate amongst cheetahs, i.e. their densities, the presence and number of other large carnivore species and/or the presence of humans in the range of cheetahs. This study might be useful to plan further parasite studies in this species and to allow for comparisons of parasite prevalence between cheetah populations in Africa and Iran.

PRO-P-26
Estimating Apicomplexan parasite exposure in Icelandic arctic foxes (*Vulpes lagopus*)

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Introduction: The arctic fox (*Vulpes lagopus*) is the only native terrestrial mammal in Iceland. The population comprises both "coastal" and "inland" fox ecotypes, with regard to food resources. While coastal ecotype foxes mainly feed on sea birds and eggs, invertebrates and marine mammal carcasses, the inland foxes feed on ptarmigans, migrating waterfowl, eggs and wood mouse. Because of the relatively low biodiversity within arctic ecosystems and the involvement of the species in both marine and terrestrial ecosystems, Icelandic arctic fox population could serve as sentinels for overall ecosystem health of Iceland.
Objectives and Methods: It was demonstrated that coastal arctic foxes have higher levels of mercury, helminth burden and richness compared to the inland populations, indicating that the two ecotypes are quite separate and distinct. However, the presence of Apicomplexan parasites has not been

reported in Icelandic foxes, yet. Using immunoblot analysis, we tested serum samples from 37 arctic foxes for the presence of antibodies to *Toxoplasma gondii*, *Neospora caninum* and *Besnoitia besnoiti.*
Results: A seroprevalence of 72.9% to *Toxoplasma gondii* antigens was found, whereas no antibodies were detected against the other two Apicomplexan parasites. There was no difference in exposure between gender and ecotype groups, however adult foxes had a significantly higher seroprevalence than juveniles (90.9% and 46.6%, respectively).
Conclusion: Compared to previous studies from Svalbard, Canada and from Medny Island (51.7%, 40% and 5%, respectively), the seroprevalence in Icelandic arctic foxes is the highest. Since the seroprevalence in human population is low (10%), further studies on the ecology and epidemiology of *Toxoplasma gondii* in Iceland are warranted.

PRO-P-27
Serum-free in vitro cultivation of *Theileria annulata*-infected lymphoblastoid cells

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The protozoan parasite, *Theileria annulata*, is the causative agent of tropical or Mediterranean theileriosis and is transmitted transstadially by *Hyalomma* ticks. *Theileria annulata* occurs in cattle, yaks, water buffalo and camels. Although *T. annulata* has been propagated *in vitro* for more than 70 years, the culture medium still contains undefined components, commonly 10-20% fetal bovine serum (FBS). Therefore, three different commercially available serum-free media (HL-1, ISF-1 and Hybridomed DIF 1000) were tested for their ability to support growth of *T. annulata in vitro* as an alternative to the use of FBS. The generation doubling times were recorded for each medium and compared to those obtained by conventional, FBS-containing RPMI1640 medium. Among the media tested, ISF-1 gave the shortest generation doubling time of an average of 35.4 hr, differing significantly from the other media. Since ISF-1-based medium is cheaper than conventional serum-containing medium, even when FBS would be replaced by newborn calf serum, serum-free cell culture media represent a cost-effective alternative. In addition, the use of serum-free culture media helps to improve animal welfare since the production of FBS is associated with ethical concerns.

PRO-P-28
In vivo effect of Coenzyme Q10 on the generation and regulation of pathologic immune responses in *Plasmodium* infection

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Cerebral malaria is a complex neurological syndrome, whose pathology is mediated by inflammatory processes triggered by the immune system of the host following infection with *P. falciparum.* Coenzyme Q10 (CoQ10) is an obligatory cofactor in the electron transport chain. The reduced form of

CoQ10 serves as a potent antioxidant additionally; CoQ10 has been identified as a modulator of gene expression, inflammation and apoptosis. However, the modulatory effects of CoQ10 during PbA infection process and risk occurrence of ECM have not been determined. In the present study we evaluated the effect of oral supplementation of CoQ10 in the host immunity and the outcome of experimental cerebral malaria caused by *P. berghei* ANKA in C57BL/6 mice. We observed that oral administration of CoQ10 prolonged the survival of mice during ECM. CoQ1O administration significantly reduced TNFα and IL-1β gene expression in brain samples and circulatory cytokines in PbA infected mice. The results also shows the ability of CoQ10 to reduce levels of CXCL9 and CXCL10 mRNA expression and cytoadhesion molecule ICAM-1 in the brain, resulting in significant reduction in the accumulation of pathogenic T cells and effector molecules in the brain and putatively improvement in the stability of the blood–brain barriers of PbA-infected mice. Furthermore, CoQ10 administration showed an enhanced upregulation of AAM gene expression. Moreover CoQ10 resulted in a significant decrease in liver aspartate aminotransferase [AST] an indicative of amelioration in liver inflammation. Our data collectively demonstrates the ameliorative function of CoQ10 on development of host inflammatory immune response, which provides an enhanced survival benefits in the murine ECM model.

Key words: Coenzyme Q10; Experimental cerebral malaria and *Plasmodium berghei ANKA.*

PRO-P-29
A new mouse model of *Giardia lamblia* infection evaluated by quantitative PCR

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Giardia lamblia (*G.l.*) is a protozoan enteropathogen that colonizes the upper small intestinal mucosa and causes a non-invasive lasting diarrheic disease in humans. It represents an import pathogen in travelers and is highly prevalent in endemic areas in many developing countries in particular in the infant population. Qualitative ELISA represents the standard method for *G.l.* detection in the routine diagnostic laboratory but this method does not allow the quantitative monitoring or comparative analysis in an infection model. Here, we established a new small animal model for *G.l.* infection using neonate mice and developed a quantitative PCR to evaluate the infection process. In contrast to adult mice that quickly cleared the pathogen, neonate animals orally exposed to *G. l.* ATCC5058 assemblage B strain GS remained chronically infected until adulthood. A quantitative PCR method was developed to quantify *G.l.* in murine fecal samples. This method enabled us to determine the parasitic load in the gastrointestinal tract and to monitor *G.l.* carriage and the process of parasite clearance during the course of the infection.

PRO-P-30
Investigations in Sarcosporidia in native rodents and treeshrews from Borneo

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Rodent species are well known reservoirs for many pathogens, some of which with zoonotic potential may have serious implications for veterinary or public health. The health status of rodent populations in tropical forests, however, is poorly studied and it is often not clear how forest conversion and fragmentation results in an increased infection of native wildlife with pathogens also found in invasive species. To better understand the possible exchange of pathogens between wildlife species and/or invasive species it is crucial to study the health status of native rodent populations. We assessed the presence of subclinical histopathological lesions in small mammals from Borneo. For this purpose, 117 individuals of native Rodentia from eight different genera (*Callosciurus, Maxomys, Niviventer, Sundamys*, *Sundasciurus* and *Leopoldamys*) and 15 individuals of three treeshrew species (*Tupaia sp*.) were collected in forest and rural landscapes in Sabah, Borneo between 2012 and 2013. Histopathological examination was carried out on tissue samples of striated muscle, lung, heart, liver, kidney, intestine and stomach.
We were able to detect specific parasitization in the skeletal muscle by the Apicomplexa protozoa *Sarcocystis sp*. in 47 rodents and 10 treeshrews. For identifying different *Sarcocystis* species we combined the evaluation of the morphologic features of the *Sarcocystis* wall using histology and electron microscopy with the molecular characterization using a partial region of the 28S rRNA suitable for phylogenetic comparison. Morphologic and genetic analyses revealed that rodents and treeshrews are mainly infected by *Sarcocystis singaporensis* and *Sarcocystis zamani*. These *Sarcocystis* species have Asian snakes, e.g. python species, as their definitive host, while invasive rats (*Rattus spp*.) are commonly reported as their intermediate hosts. Our study provides evidence that *S. singaporensis* and *S. zamani* use an even broader range of intermediate host species than anticipated so far. The infection of native small mammal species in high prevalence ensures the continuation of the parasite"s lifecycle irrespective of the predators" habitat choice as pythons are known to roam in urban areas as well as deep into the tropical forests.

PRO-P-31
Are *Toxoplasma gondii* late embryogenesis abundant domain-containing proteins (TgLEAs) suitable for serological identification of infection by oocysts?

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Introduction: The genome of *Toxoplasma gondii* encodes small oocyst specific proteins, annotated as "late embryogenesis abundant domain-containing proteins" (TgLEAs). Usually such proteins don’t have

a defined secondary structure under physiological conditions but can adopt ordered structures under specific stress conditions like desiccation or low temperatures. Thereby they protect organelles and proteins from these insults. One of the TgLEAs (TgLEA-850) used here has already been reported by others as an oocyst specific antigen, enabling specific detection of infection by oocysts.
Objective: Since TgLEAs are known to be oocyst-specific antigens we wished to further explore the suitability of recombinant TgLEAs to differentiate infections in individuals caused by *T. gondii* oocysts vs. tissue cysts.
Methods: TgLEA-proteins (TgLEA-850, -870, -880) were expressed in *Escherichia coli* and purified. To test whether mice are able to mount an antibody response against TgLEAs, BALB/c mice were subcutaneously immunised with recombinant TgLEAs mixed with adjuvant. In addition, groups of C57BL/6 mice were orally infected with varying doses of *T. gondii* tissue cysts or oocysts. Sera sampled from 11 - 36 days p.i. were used to monitor successful infection by testing for tachyzoite-specific antibody responses by ELISA.
Results: Mice immunized against TgLEA-850 or TgLEA-880 developed a weak or no IgG antibody response, respectively, against these proteins, while those inoculated with TgLEA-880 responded strongly in immunoblot analyses. Neither mice infected with oocysts nor those inoculated with tissue cysts developed a measurable antibody response against any recombinant TgLEA as examined by immunoblot. Currently, these results are verified by ELISA.
Conclusions: Mice immunized with recombinant TgLEAs are able to mount at least weak antibody responses against these proteins. Because none of the mice orally infected with *T. gondii* developed antibodies, TgLEAs seem to be of limited suitability for diagnostic purposes.

PRO-P-32
Bumped Kinase inhibitor 1294 and its effect on protein expression and localization in *N. caninum*.

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The bumped kinase inhibitor BKI-1294 affects the activity of calcium-dependent protein kinase 1 (*Nc*CDPK1), and interferes in tachyzoite invasion and intracellular proliferation. However, even at high concentrations, BKI-1294 does not exert parasiticidal effects *in vitro*, but induces the formation of large schizont-like multinucleated complexes (MNCs), which remain viable for extended periods of time. Within these MNCs parasites are blocked during cytokinesis. *In vivo,* BKI-1294 treatment largely inhibited *N. caninum* infection in the CNS, and blocked vertical transmission in *N. caninum*-infected pregnant mice.
The distribution of *Nc*CDPK1 in BKI-1294 treated and untreated *N. caninum* tachyzoites was studied by immunofluorescence and immunogold-electron microscopy. In addition, the expression and localization of several tachyzoite and bradyzoite proteins (SAG1, BAG1, IMC1 and others) was investigated by RT-PCR, western blot and immunofluorescence during the formation of MNCs and subsequent recovery phase after drug release. In intracellular tachyzoites, *Nc*CDPK1 is distributed all over the cytoplasm. Once parasites reach the extracellular space, the localization shifts towards the apical part, and *Nc*CDPK1 is associated with the supellicular membrane, few micronemes, and the conoid. This apical shift is not inhibited by the presence of BKI-1294. During BKI-1294 treatment of *N.*

caninum infected fibroablasts, MNCs exhibit a deregulated mRNA and antigen expression pattern, including a diminished expression of SAG1 and NcCDPK1, but newly formed daughter zoites are formed within these MNCs as visualized by continuous IMC1 expression and a lack of SAG1 surface staining. 6-8 days after the drug is removed, newly formed tachyzoites with SAG1 surface antigen expression emerge directly from the MNCs. We hypothesize that MNC formation and associated deregulated antigen expression contributes to protective immunity, thus explains the excellent efficacy of this compound in vivo. The results of this study could lead to a better understanding of the cross-talk between chemotherapy and immunity in experimental murine neosporosis.

We acknowledge financial support by SNF [grant No. 310030_165782], PHS, NIH [grants R01 AI 111341 and R01 HD 080670], and USDA [grant 2014-67015-22106].

Vectors, entomology and acarology

VEA-P-01

Establishment of an *in vitro* feeding system for *Ixodes* spp. to test vector competence of diverse German *Ixodes* populations for *TBEV*

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Despite many years of research, the current state of knowledge concerning the vector competence of ticks for the *tick-borne encephalitis virus* (*TBEV)* is incomplete. For investigation of virus replication and transmission, the establishment of an *in vitro* feeding system was necessary. After modification of a system by Krull *et al.* (2017), further parameters to improve the feeding efficiency and to adapt the system to BSL-3 conditions have been tested.
Ixodes nymphs were fed artificially over a period of 10 days. To investigate the impact on tick attachment, blood was changed in different time intervals, feeding chambers were moved for steady turbulence of blood, different incubation temperatures as well as different olfactory stimuli have been compared. Blood change twice a day resulted in an increased attachment rate (48%). Furthermore, 70% of these ticks completed blood meal and 6 of those nymphs molted to adult stage. In comparison, only 18% tick attachment and 55.6% engorgement was observed with a blood change once a day. Attachment of ticks receiving fresh blood twice a day was 26% and 23% of these could complete blood meal. Fewer tick attachment (16%) was observed in experiments where blood was replenished once a day but with continuous movement. However, the feeding rate of these ticks (25%) was slightly higher. At an incubation temperature of 36 °C, 64% ticks attached to the membrane, of which 59.4% were fully engorged. Metamorphosis could be observed in 5 of these nymphs. In the comparison group, incubated at 32 °C, 48% of ticks were attached, from which 37.5% were fully engorged. Six of these ticks have molted. In conclusion, a preference for an incubation temperature of 36 °C to 32 °C, blood change twice a day and an olfactory stimulus with dog hair could be observed.

Krull, C., Böhme, B., Clausen, P. H., Nijhof, A. M. (2017): Optimization of an artificial tick feeding assay for *Dermacentor reticulatus*. Parasites and Vectors, 10: 60

VEA-P-03

Outbreak of tick-borne encephalitis transmitted by unpasteurized transmitted by unpasteurized goat's milk and cheese in Germany, June 2016

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In June 2016, a cluster of two out of four members of a hiking group developed tick-borne encephalitis (TBE) in the Federal State of Baden-Württemberg in southwestern Germany. Initial investigations revealed a possible link with raw goat milk andor goat cheese sold by a goat farm. The respective region has not been known as TBE-endemic so far. We performed an outbreak investigation in order to confirm the source of infection and extension of the outbreak. None out of 32 other consumers showed IgM antibodies against TBEV(TBEV) at three to eight weeks after consumption, and none of the 27 notified TBE cases within the state reported to have consumed raw goat milk or cheese from the suspected farm. Five of 22 cheese samples (23%) from 5 batches were RT-qPCR positive for TBEV-genome, and two of the five samples were confirmed by virus isolation, indicating the viability of TBEV in cheese. Nine out of the 45 (20%) goats had neutralizing anti-TBEV antibodies, two with a high titer, indicating a recent infection.
One adult female *Ixodes ricinus* tick out of a total of 412 ticks was also RT-qPCR-positive, and sequencing of the E gene directly from the extracted tick nucleic acid confirmed TBEV. Phylogenetic analyses of both the tick and cheese isolates show a 100% homology of amino acids in the E gene and a close phylogenetic relation to TBEV strains from Switzerland and Austria.
To the best of our knowledge, we report the first isolation and preliminary genetic characterization of TBEV from goat cheese and ticks of a food-related TBEV outbreak.

VEA-P-04

Avian malaria on Madagascar: bird hosts and putative vector mosquitoes of different *Plasmodium* lineages

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Question: Avian malaria occurs almost worldwide and is caused by Haemosporida parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*). Vectors such as mosquitoes, hippoboscid flies or biting midges are required for the transmission of these parasites. There are few studies about avian malaria parasites on Madagascar but none about suitable vectors.
Methods: To identify vectors of avian *Plasmodium* parasites on Madagascar, we examined head, thorax and abdomen of 418 mosquitoes from at least 18 species using a nested PCR method to amplify a 524 bp fragment of the haemosporidian mitochondrial cytochrome b gene. Sequences obtained were then compared with a large dataset of haemosporidian sequences detected in 45 different bird species (n = 686) from the same area in the Maromizaha rainforest.
Results: Twenty-one mosquitoes tested positive for avian malaria parasites. *Haemoproteus* DNA was found in nine mosquitoes (2.15%) while *Plasmodium* DNA was found in 12 mosquitoes (2.87%). Seven distinct lineages were identified among the *Plasmodium* DNA samples. Some lineages were also found in the examined bird samples: *Plasmodium* sp. WA46 (EU810628.1) in the Madagascar bulbul, *Plasmodium* sp. mosquito 132 (AB308050.1) in 15 bird species belonging to eight families, *Plasmodium* sp. PV12 (GQ150194.1) in eleven bird species belonging to eight families and *Plasmodium* sp. P31 (DQ839060.1) was found in three weaver bird species.
Conclusion: This study provides the first insight into avian malaria transmission in the Maromizaha rainforest in eastern Madagascar. Five *Haemoproteus* lineages and seven *Plasmodium* lineages were detected in the examined mosquitoes. Complete life-cycles for the specialist lineages WA46 and P31 and for the generalist lineages mosquito132 and PV12 of *Plasmodium* are proposed. In addition, we have identified for the first time *Anopheles mascarensis* and *Uranotaenia* spp. as vectors for avian malaria and offer the first description of vector mosquitoes for avian malaria in Madagascar.

VEA-P-05

Detection of Crimean-Congo Hemorrhagic Fever Virus in hard Ticks in one of the Southwestern provinces of Iran

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Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne viral zoonotic agent in the family Bunyaviridae, genus Nairovirus. The virus is transmitted to humans through tick-bite or by contact with blood or tissues from infected patients or viremic livestock. Results: This study was carried out to investigate the infection of species belonging to Ixodidae family of ticks in Khuzistan province, Iran. In total, 102 ticks stick to the animals' body were collected: 57, 19 and 26 hard ticks in spring, summer

and autumn, respectively. The ticks were classified into two genera including: Rhipicephalus (86.3%), Hyalomma (13.7%). RT-PCR showed CCHFV in 6.9% (7 of 102) of tick samples. Conclusions: According to the seasons, the infected ticks were different. Four of 56 Rhipicephalus sp. Were infected in spring. CCHFV was detected in 3 of 14 Hyalomma ticks in summer and 1 of 1 Hyalomma sp. in autumn. In contrast with other articles that mentioned Hyalomma ticks are the principal source of human infection, in this survey we showed Rhipicephalus ticks are the main vector of CCHFV during spring and Hyalomma ticks are the main vector of virus in other seasons. So, distribution, prevalence and infection of hard ticks were varied in different seasons. Keywords: CCHFV, Rhipicephalus, Hyalomma, RT-PCR

VEA-P-06
Study on Ticks infection (Argasidae and ixodidae) to Anaplasmosis eastnorth Iran

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Background: Anaplasmosis, as an endemic disease in most tropical and subtropical regions is caused by a kind of bacterium called Anaplasma. While fever, nemia, weakness, constipation, depression, dehydration are the most important signs of the disease, it's mortality rate ranges between 5 to 40%. As an obligatory gram negative bacterium, Anaplasma has a complete lifecycle in ticks, especially Ixodidae. Due to deficiency of documented information about Anaplasma species in North Khorasan province of Iran, we set out to determine the prevalence of the infection rate in this zone of the country.
Methods: North Khorasan is located in northeast of Iran. Ticks were collected from the base of tails, ears, neck, and back of the bodies as well as around genitalia of livestock by forceps. The samples were kept in appropriate temperature and humidity and then transferred to the Laboratory. After identification of species, Tick DNA was extracted and Nested-PCR reaction was done to detect Anaplasma infection.
Results: Totally, number of 395 livestock was checked in this study. A total number of 506 collected ticks were belonged to Rhipicephalus, Hyalomma, Dermasentor, Haemaphysalis and Boophilus Genera. Tick's relative abundance in mountain to plain was 3 to2 and Rhipicephalus was the most prevalent genus. Nested-PCR revealed that 3.2% of the ticks were infected by Anaplasma spp. The infection was more prevalent in Dermacentor marginatus.
Conclusion: The results of this study connfirmed the significant role of hard ticks in prevalence of the infectious rate in livestock of the region. Moreover, the data revealed that precise seasonal and consecutive investigations about the infestation of ticks and tick-borne diseases must be applied to control the rate of Anaplasmosis and zoonose diseases in North Khorasan province of Iran.
Key word: Tick, Anaplasma, Khorasan, Iran

VEA-P-07
Resting sites of mosquitoes in Germany

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Most common mosquito traps predominantly collect host-seeking females. On the other hand, sampling of resting mosquitoes might give us information on males and gravid/blood-fed females. In particular, females require resting sites to digest their blood meal. Thus, the environment (e.g. microclimate) during resting period may influence mosquitoes' gonotrophic cycle and their ability to transmit diseases. In this study, we compared natural and artificial resting sites to get further insights into resting site preferences and to develop methods to catch a wide range of different mosquito species.
Between May and October 2017, 30 study sites in Southern Germany were studied. These sites represent different types of natural wetland habitats. At each site, nine artificial resting sites (= garden pop-up bags, 76 l) were installed in a tree at three different heights (0, 2, and 5 m). Additionally, different natural microhabitats around the artificial resting sites were directly sampled. Mosquitoes were collected using a battery powered aspirator.
The results showed clear species-specific preference for the different types of resting site. Mosquitoes of the genus *Aedes* were predominantly found in natural resting sites, whereby species of the genus *Culiseta* and *Anopheles* were dominant in the artificial resting site. More specimens were sampled at ground level for both, artificial and natural resting sites, while fewer mosquitoes were sampled at the elevated resting sites.
Garden pop-up bags are useful tool to catch a wide range of mosquito species. However, natural resting sites should be integrated to catch the entire spectrum of species. Further research is required to understand the role of resting site availability for mosquito ecology and the influence of resting microhabitats on the time period of pathogen development and blood meal digestion.

VEA-P-08
Origins of recently emerged foci of the tick *Dermacentor reticulatus* in Poland inferred from molecular markers

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Question: Ornate dog tick *Dermacentor reticulatus* is vector of several blood parasites, including *Babesia canis*, a causative agent of babesiosis. The geographical range of *D. reticulatus* in Europe is discontinuous with a gap separating eastern and western macroregions. New foci observed in several locations in western and central Europe were considered an expansion of the western population, including foci in western Poland.

Methods: In this study we used molecular markers to identify the origins of these foci, and we compared their genetic polymorphism to *D. reticulatus* collected in sites situated within the eastern population. Altogether, six local tick populations were studied, including two populations from central and NE Poland, two populations from western Poland, one site from Ukraine (Kiev area) and one site from Germany (Potsdam area), spanning the distance of about 1200 km.
Results: The overall polymorphism in mt 16S rDNA was low, and all sites from the western population shared the same haplotype suggesting the expansion in this area. In the marker 5.8S- ITS2 rDNA we found no differences in polymorphism between sites from eastern Poland (eastern population), and newly emerged foci in western Poland considered a putative expansion zone of the western population. However, the sites from western Poland differed considerably from nearby German site (Potsdam area). Our results show that foci in western Poland could not have originated from *D. reticulatus* from the western population, as previously thought. We found that the state border following river hinders considerably gene flow between adjacent sites what suggest that natural dispersal of *D. reticulatus* by wildlife is unlikely, and the emergence of new foci should rather be contributed to human-associated dispersal.
Conclusions: We propose that livestock and pets travelling with their owners are the most probable source of new foci, and they can easily transfer ticks within a country but not between countries.

Figure 1

Kloch et al., Vet Parasitol. 2017

VEA-P-09
Dispersal of *Culicoides* in farm environments
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Since the severe Bluetongue epidemic in 2006, farmers in Europe increased efforts to reduce breeding sites of the BTV-vector *Culicoides* (Diptera, Ceratopogonidae). As effective measures need a good understanding of *Culicoides* host seeking behavior, we used non-attractant sticky traps to study the dispersion patterns of biting midges around different cattle farms located in Lower Saxony. Sticky traps were exposed to cover approx. 6% of all open entry routes (windows, doors) in order to sample adult *Culicoides* flying into and out of dairy cattle stables. *Culicoides* were caught for 48 h during four consecutive weeks in late summer 2016 and in spring 2017. At the same time, abiotic parameter (wind speed, wind direction, and temperature) were recorded and light suction traps were placed with an 24 h offset to the sticky traps inside and outside the stable.
The number of individuals and the flight direction of biting midges differed considerably between farms and some sides of the stables were preferred over other sides on one of the farms. Here, *Culicoides* entered and left the stable along the main wind direction. However, no clear preferences occurred on the other farm. According to our preliminary results, we assume that wind direction might influence the dispersal of *Culicoides.* However other factors also seem to have an effect but are still unknown.

VEA-P-10
Flourishing in germs: Deciphering the role of bacteria in development of the malaria vector *Anopheles coluzzii*

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In Africa, *Anopheles gambiae* mosquito is the major malaria vector. Despite this vector harboring a diverse microbiome, no single bacteria species has been identified as its obligate symbiont. However, it is known that bacteria are essential for *Anopheles* and other mosquito development, as axenic mosquitoes fail to develop beyond the first larval stage. Development is restored by association with various bacterial species. Therefore, the factors that promote mosquito development must be conserved and produced by various bacterial species. Currently, the underlying mechanism behind the essentiality of bacteria for mosquito development remains unknown. To determine why mosquitoes need bacteria, we are performing a systematic screen using a whole genome knock out library of *E. coli* mutant strains (Keio collection). Our preliminary results confirm that *E. coli* supports development of axenic larvae. Our screen should identify: (1) *E. coli* mutants that do not rescue larval development, and (2) microbial compounds and metabolites that are required for larval development. 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*.

VEA-P-11
Developing new traps for catching blood-hungry *Simulium damnosum* in Cameroon

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Introduction: There is a need for replacing the human fly-collector by an automatic trap for monitoring the success of ongoing onchocerciasis control.
Objectives: The *Simulium*-trap should be specific, i.e. attract and catch only blood-hungry female flies record the time of landing, measure and automatically store environmental parameters, and finally catch and preserve the flies in such a condition that they can still be dissected for parous and filarial infection rates.
Materials & Methods: A new trap was developed, based on a cube and employing olfactory (CO2) and visual cues. *Simulium* flies attracted are sucked in by a 12-V driven ventilator. Environmental parameters were recorded on an Arduino microcontroller.
Results: This new trap, which we name "sucking-cube", attracts *Simulium* flies by colour and the smell of CO2 locally produced in a fermenter. It consists of a square cube of 53 cm length with openings on the sides and below. The flies are sucked in by the ventilator and directed towards an easily removable catching chamber, which is automatically changed every hour.
Field trials were carried out along the *Simulium* breeding-river Vina du Sud near Ngaoundéré (North-Cameroon). In two weeks, more than 2.000 *Simulium damnosum* s.l. flies were caught. The trap was tested against other types of *Simulium* fly-traps: The "Magic flyboy"1), the "Esperanza Window trap"2) both designed to catch blood-hungry females and the "Bellec-plates"3), which mimics a breeding site and thus primarily attract gravid females.
Conclusion: The "sucking-cube" is a new trap, which has potential to replace the human standard fly-collector.

Supported by DFG (Re1536/5ff) and BW-Stiftung (BW-plus)

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Fig: Traps

Figure 1

VEA-P-12

Prospection of *Simulium* flies in Tunisia

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Introduction: Despite the important role of Simulium flies in the transmission of parasites and their nuisance as a biting pest, little is known about the occurrence of Simulium flies in Tunisia since 1970, when Bailly-Choumara and his team made a short field trip through Northern part of the country. Objectives: The aim of this study was to search for *Simulium* breeding sites and to identify the present species. Thus, an evaluation of their role as vectors in this country and the potential risk they can represent for animals there as well as in Europe will be possible.
The Barcode of Life Data (BOLD) System is to be supplemented by the sequences of a barcode region of multiple *Simulium* flies.
Methods: Two short surveys were carried out in Northern Tunisia in December 2016 and 2017. *Simulium* larvae and pupae were collected and stored in 70% EtOH. The flies were examined morphologically in the National Veterinary School of Sidi Thabet and the University of Tübingen. Molecular species determination using the barcoding approach is currently taking place at the University of Tübingen. The protocol targets a 658bp region in the COI-gene, as described by Hernandez-Triana et al. (2015).
Results: 11 breeding sites were located. So far, the same species were identified as already found in 1970: *Simulium latipes, S. wilhelmia, Odagmia ornata* and *Boophtora erythrocephalum*. However, we expect new results from the molecular examination of the samples, while delivering new data for the BOLD System.
Conclusion: *Simulium* flies are common in Northern Tunisia. Regarding the dense lifestock farming, it is likely that these flies play an important role as vectors. Further studies on *Simulium*-transmitted diseases like the filarial parasite *Onchocerca lienalis* in cattle are planned to take place, investigating the need of vector control.
Due to its geographical position, Tunisia bridges between Africa and Europe. With a view to filarial parasites, it shall be interesting to find out whether these belong to species and strains commonly found in Africa or Europe or in both continents.

Figure 1

Figure 2

Veterinary parasitology

VPA-P-01

The apicomplexan parasite *Neospora caninum* triggers neutrophil extracellular traps in bottlenose dolphin (*Tursiops truncatus*) PMN

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Background: *Toxoplasma gondii* and *Neospora caninum* are closely related apicomplexan parasites which can cause infections in many domestic animals, but also in wildlife animals including bottlenose dolphins (*Tursiops truncatus*). So far, no evidence is known on early innate immune reactions against

N. caninum in dolphins. Neutrophil extracellular trap (NET) formation is an effective host defense mechanism of polymorphonuclear neutrophils (PMN) acting against protozoan parasites *in vitro* and *in vivo*.
Objective: Confirm the capacity of vital *N. caninum* tachyzoites to trigger NETosis in cetacean-derived PMN.
Methods: Visualization of NETs was achieved by SEM analysis. The identification of classical NETs components [i. e. histones (H1, H2A/H2B, H3, H4), neutrophil elastase (NE), myeloperoxidase (MPO) and pentraxin (PTX)] was performed via immunofluorescence-based analysis. Extracellular DNA staining via Pico Green®- assays was used to illustrate and quantify NET formation. *N. caninum* tachyzoites were exposed to PMN at different ratios, time spans and in presence and absence of DNase I and the NADPH oxidase (NOX) inhibitor DPI.
Results: *N. caninum* tachyzoites revealed as potent inducers of cetacean NETosis. SEM analyses demonstrated NET filaments and diffuse structures being released by dolphin PMN and entrapping tachyzoites. Co-localization of extracellular PMN-derived DNA networks with histones (H1, H2A/H2B, H3, H4), NE, MPO and PTX confirmed the molecular characteristics of classical NETs that additionally proved as a dose-dependent process. As expected, DNase I treatments resolved NETs and diminished NET formation following DPI treatments confirmed NOX-dependency of dolphin-triggered NETosis.
Conclusions: NETosis was identified as an effective mechanism bottlenose dolphin PMN directed against *N. caninum* tachyzoites. Thus, the current study confirmed the relevance of this mechanism in the cetacean system.

VPA-P-02
Detection of *Neospora caninum*-specific antibodies in breeding bitches

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Background: *Neospora caninum* is an obligate intracellular apicomplexan parasite which can cause abortive infections in ruminants. Canines are known as the definitive hosts which excrete oocysts into the environment. Importantly, in the canine system *N. caninum* infections may also be transmitted intrauterine from the bitches to the pubs.
Objective: Determine the presence of *N. caninum*-specific antibodies in German breeding bitches.
Methods: A total of 218 serum samples of German breeding bitches were analyzed using a commercial indirect enzyme-linked immunosorbent assay (ELISA). To corroborate canine infections, positive sera were additionally tested via immunoblotting techniques. The owners of seropositive bitches were interrogated on breed, age, habitat, type of dog population, vaccine status, feeding habits and the presence of reproductive disorders.
Results: *N. caninum*-specific antibodies were found in 16/218 (7.33%) bitches. Immunoblotting analyses confirmed all ELISA-positive samples proving infections in these animals. The ages of the

seropositive animals ranged between two and seven years. Three of them were kept in kennels, one was also a hunting dog and the rest were household animals. Four seropositive bitches presented merely one gestation; the other 12 bitches recorded multiple pregnancies. Fourteen seropositive animals were regularly vaccinated and six individuals consumed raw meat.
Conclusions: This is the first report on *N. caninum* infections in German breeding dogs, confirming an overall low seropositivity. Further epidemiological studies are necessary to complement the current results on neosporosis in this particular canine population in Germany.

VPA-P-03
First molecular detection and phylogenetic analyses of *Neospora caninum* from naturally infected sheep in North Africa

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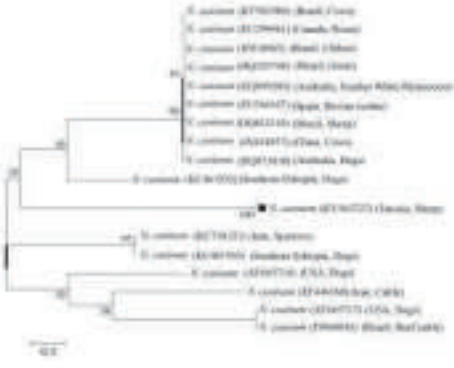
Introduction: *Neospora caninum* is a protozoan parasite from the phylum Apicomplexa. This parasite is as one of the major causative abortive agents in the dairy cattle industry. Moreover, abortions in sheep due to *N. caninum* have been reported by several studies (Moreno et al, 2012). The objective of this study was to estimate the molecular prevalence and phylogenetic analyses of *N. caninum* in Tunisian sheep.
Methods: A total number of 198 meat samples were collected from slaughtered ewes and tested for the presence of *N. caninum* ITS1 gene using PCR followed by sequencing of some PCR products. A phylogeneyic tree was then constructed to compare the partial sequences of the ITS1 gene with GenBank sequences.
Results: The overall *N. caninum* infection prevalence rate in sheep was 10.6±4.3% (21/198). The infection rate was significantly higher for animals aged over one year (19.4±9.1%) when compared to those under one year of age (5.6±4) ($p<0.001$). The highest prevalence was observed in North Béja locality (31.2±16.1) ($p<0.001$) (Fig. 1). Compared to Barbarine (6.8±4.5%) and Cross-bred animals (0%), Noire de Thibar was the most infected sheep breed (31.7±14.2%) ($p<0.001$). Comparison of the partial sequences of the ITS1 gene revealed 96-98% similarity among our *N. caninum* amplicon and these deposited in GenBank (Fig. 2).
Conclusions: To our knowledge this is the first molecular study and phylogenetic analysis of *N. caninum* in sheep in North Africa. Our results indicate the meat harbour *N. caninum* cysts and can contribute to a better evaluation of *N. caninum* infection and the associated abortions in sheep.

Figure legends:
Fig 1: Molecular *Ne. caninum* infection prevalence of slaughtered sheep North-West Tunisia.
Fig 2: Phylogenetic tree of ITS1 rDNA gene for *Neospora caninum* isolated from sheep.

References: Moreno, B. (2012). Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. Vet. Parasitol., 187, 312–318.

Figure 1

Figure 2



VPA-P-04
Microsatellite analysis by FLA provides markers for strain discrimination of geographically close isolates of *Cystoisospora suis*

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Microsatellites are short repetitive DNA sequences of two to six repeats interspersed in the genome which display a rapid mutation rate and consequently show high variation between individuals or populations. They have been used to characterise population diversity and structure and the level of

variation between different isolates of a variety of organisms, including apicomplexan protozoa. Currently nothing is known about the genetic variability and population structure of *Cystoisospora suis*, the causative agent of piglet coccidiosis, and we made use of the recently available genome of *C. suis* (strain Wien-I) to generate sets of primers for the amplification of microsatellite regions (ca. 400 bp) in non-coding or intergenic regions to evaluate the applicability of fluorescence-labelled primers to analyse amplicon length variation at high resolution using capillary electrophoresis (CE) technique. Two phenotypically characterised isolates (Wien-I, toltrazuril susceptible; Holl-I, toltrazuril resistant) and three field isolates from Europe were compared in conventional PCR to evaluate the applicability of the method. Eight primer pairs amplified bands of the expected size for Wien-I and were labelled for CE analysis. High resolution CE of the amplicons revealed high diversity of the analysed strains, with differences even between two strains from neighbouring swine farms. In follow-up studies, adaptation of the PCR assay to multiplexing and amplification of small DNA quantities will provide a cost-effective tool to analyse field strains to reveal geographic diversity and possibly assignments to phenotypic traits. Stability of markers across stages and generations of the parasite must also be addressed.

VPA-P-05

Gastrointestinal parasites of wild Antillean manatees (*Trichechus manatus manatus*) in Colombia and first phylogenetic analysis of their trematodes and *Eimeria* species

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Introduction: The Antillean manatee (*Trichechus manatus manatus*) is the only herbivorous aquatic mammal inhabiting salty, brackish and fresh tropical waters, rendering it a unique representative of the sirenids. Wild manatee populations are still endangered, mainly by human population growth in coastal areas, pollution, habitat degradation and poaching. The assessment of parasitic burden and epidemiological studies represent a relevant factor for conservation programmes on wild Antillean manatee populations.
Objectives: In the current study we performed a coprological and phylogenetic analysis of the gastrointestinal parasites of Antillean manatees in Colombia.
Materials & Methods: Faecal samples from 73 Antillean manatees were collected at the Caribbean wetlands of Colombia and analysed via sodium acetate acetic acid formalin (SAF)-technique, sedimentation, flotation and carbolfuchsin-stained faecal smears. In addition, *Cryptosporidium*- and *Giardia*-specific coproantigen-ELISAs were performed. Faecal DNA isolation and parasite-specific PCR were conducted for phylogenetic analyses.
Results: Overall, 73 % of the samples were parasitized. Apicomplexan parasites revealed as the most prevalent ones. Sorted by prevalence, we found the following parasites in manatee faecal samples: *Eimeria nodulosa* (46.5 %), two undetermined *Eimeria* spp. (42.4 %), followed by the trematodes

Chiorchis fabaceus (35.6 %) and *Nudacotyle undicola* (4.1 %), and the diplomonadid *Giardia* spp. (1.4 %). In addition, partial DNA sequences of the ribosomal RNA genes of the trematodes and *Eimeria* spp. were amplified by PCR, sequenced and used to infer phylogenetic relationships.
Conclusion: Antillean manatees proved considerably infected with different parasite species. Besides the first report on *Eimeria* and *N. undicola* infections in Antillean manatees this study represents the first molecular analysis of manatee parasites.

VPA-P-06
Analysis of molecular mechanisms involved in *Besnoitia besnoiti*-mediated NETosis

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Background: The apicomplexan parasite *Besnoitia besnoiti* is the causative agent of bovine besnoitiosis, a disease that affects mainly the productivity and fertility of cattle. NETosis represents an important innate effector mechanism of neutrophils acting especially against large pathogens. Recently, neutrophil extracellular trap (NET) release was reported in response to *B. besnoiti* tachyzoites.
Objective: In this study we analyzed the role of PMN-derived autophagy and of reactive oxygen species (ROS) production in *B. besnoiti*-induced NETosis.
Methods: Bovine neutrophils were isolated from blood by Biocoll® separating solution. *B. besnoiti* tachyzoites were propagated in MDBK-based cell culture. Co-cultures of bovine PMN and tachyzoites were performed at MOIs of 1:4. To analyze the role of autophagy, neutrophils were pretreated in a dose-dependent manner with rapamycin (stimulator of autophagy) and wortmannin (inhibitor of autophagy) prior to tachyzoite exposure. Intracellular and extracellular ROS were measured using spectrophotometric methods. NETs were visualized by scanning electron microscopy (SEM) and by immunofluorescence using co-staining of extracellular DNA (sytox orange) and histones or neutrophil elastase (NE) via specific antibodies. Autophagosomes were visualized by anti-LC3B antibodies via immunofluorescence and confocal microscopy.
Results: SEM and immunofluorescence-based analyses on PMN-derived histones and NE in extracellular DNA structures confirmed the significant induction of NETosis by *B. besnoiti* tachyzoites. However, neither extracellular nor intracellular levels of ROS were found enhanced after kinetic analyses of bovine PMN/*B. besnoiti*-tachzoite co-cultures denying a key role of ROS in parasite-triggered NETosis. In addition, both, rapamycin and wortmannin treatments failed to influence tachyzoite-triggered NETs.
Conclusion: Overall, *B. besnoiti*-mediated bovine NETosis appears independent of ROS production and PMN-derived autophagy.

VPA-P-07

Development of Pyrosequencing Based Assays for Benzimidazole Resistance Detection in *Heterakis gallinarum* and *Ascaridia galli*

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Introduction: Following recent changes in husbandry systems for poultry in Europe from cage to e.g. free range systems increased prevalence of *Ascaridia galli* and *Heterakis gallinarum* have been encountered. Benzimidazole (BZ) resistance is widespread in several parasitic nematodes of ruminants and horses where it correlates with the presence of three single nucleotide polymorphisms (SNPs) in the β-tubulin isotype 1 gene. However, to date no reports of BZ resistance in *A. galli* and *H. gallinarum* have been published.
Objectives: This study aimed to (i) identify β tubulin isotypes of *A. galli* and *H. gallinarum*, (ii) develop pyrosequencing assays for the detection of SNP at codons 167, 198 and 200 of the β-tubulin genes, and (iii) determine the frequency of BZ resistance associated SNPs in adult worms and pooled eggs from the field samples.
Materials & Methods: Degenerated primers were used to obtain partial cDNA fragments that were completed using rapid amplification of cDNA ends (RACE) and cloning of full-length cDNAs. Pyrosequencing assays were developed using synthetic fragments containing the relevant SNPs.
Results: For both nematodes, only cDNAs representing a single β-tubulin isotype were obtained using degenerated primers. The 9 *A. galli* sequences were 100% identical to a previously published sequence while the 40 new *H. gallinarum* sequences were >99% identical to each other and 88% identical to the *A. galli* sequence. Pyrosequencing assays for all three SNPs in each β-tubulin gene were developed and evaluated, showing high accuracy and a low background to detect potential resistance-associated SNPs.
Conclusion: The developed assays offer the tools to screen for the presence and to quantify the relative frequency of BZ resistance associated SNPs in populations of important poultry parasites. The assays will allow to detect the development of resistance in an early phase before it becomes clinically apparent. This offers the chance to implement measures to counteract further selection before resistance becomes widespread.

VPA-P-08

Fox in trouble. Tick-borne microparasites affecting red fox (*Vulpes vulpes*) in Poland.

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Red fox (*Vulpes vulpes*) is the most abundant carnivorous species in both natural and urban areas, thus may serve as an ideal reservoir host for many pathogens infective to humans or dogs. Foxes are exposed to infestation with many tick species and may be infected with a range of tick-borne pathogens (TBPs). Despite the detection of TBPs in foxes, the impact of infections on this host species was never studied.
The aims of present study were: (i) to investigate potential of red fox as reservoir of tick-borne microparasites in Poland and (ii) to evaluate pathological changes caused by these infections in red foxes.
Pups of red fox (n=14) were live-trapped to collect blood samples and perform blood smears. Foxes killed in road accidents or provided by hunters (n=150) were subjected to autopsy. Internal organs were weighed. Genomic DNA was extracted from spleen and blood samples. Specific primers were used to amplify fragments of 18S rDNA of *Babesia* spp. (~520 bp) and *H. canis* (~625 bp). Positive amplicons were sequenced and compared with known sequences from GenBank using BLAST tool. Results of molecular analyses and autopsy findings were subjected to statistical tests.
Molecular analysis showed high prevalence of *H. canis* (18.7%) and *B. vulpes* infection (24/150= 16%). Three samples tested positive for *B. canis* DNA (2%), from foxes living in mazowieckie voivodship. All but one pups harbored *H. canis* (93%) and *B. vulpes* was detected in one individual (7%). Both spleen and kidney mean weights were higher in animals infected with *B. vulpes*. Index of spleen/ body mass was significantly higher in animals infected with *B. vulpes*.
Conclusions. Red fox constitutes the important reservoir host of *H. canis* and *B. vulpes* in Poland. Infection with *B. vulpes* may negatively impact health status of foxes.

Acknowledgements: The study was funded by the National Science Centre (NCN) grant Sonata Bis 2014/14/E/NZ7/00153.

VPA-P-09
Retrospective evaluation of vector-borne infections in dogs imported from endemic regions (2007 – 2015)

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Introduction: Canine vector-borne diseases gained in importance in Germany due to climatic changes, growing tourist traffic and the increased import of dogs from abroad. Endemic regions for pathogens as Leishmania (L.), Hepatozoon (H.), Ehrlichia (E.) and Dirofilaria (D.) are the Mediterranean area and Southeastern Europe. A variety of species of Anaplasma (A.) and Babesia (B.) are found throughout Europe. **The objective** of this retrospective study was to determine the prevalence of vector-borne infections in dogs imported from endemic regions. **Material and methods:** The examination results of 345 patients of the Small Animal Clinic FU Berlin between 01/2007 and 12/2015, which were brought into Germany from endemic regions (13 countries in the Mediterranean area, 4 in Southeastern Europe), were evaluated. A total of 1368 tests were sent to external laboratories (576 direct / 792 indirect methods of detection). **Results:** The positive results were distributed as follows: A. platys 1/21 dogs (4.8%; PCR), E. canis 45/278 (16.2%; PCR 8/68; IFAT 43/257), B. spp. 3/98 (3%, genus specific PCR), B. canis 22/213 (10.3%; IFAT/ELISA 22/214), B. gibsoni 0/13 (IFAT), B. spp./H. spp. 0/8 (PCR), H. canis 3/28 (10.7%; PCR), L. infantum 66/314 (21%; PCR 20/79; IFAT/ELISA 63/308), D. immitis 8/156 (5.1%; ELISA), microfilariae 5/23 (21.7%; PCR). The Knott's test was positive in 7/95 (7.4%) dogs. Mixed infections were detected in 27/345 dogs (7.8%). **Conclusion:** Imported dogs are often infected with one or more vector-borne infectious agents. 34.8% of the tested dogs were positive for at least one pathogen. Potential owners of imported dogs should be informed and all dogs tested for vector-borne infections.

VPA-P-10
Molecular infection prevalence of ruminants Tunisian meat by three protozoa: *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystic* spp.

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Ruminants' meat is the main food Human protein source in Tunisia (mainly lamb and beef meat). This meat can be contaminated by two zoonotic protozoa: *Toxoplasma gondii* and *Sarcocystis hominis*. *Neospora canimum* is a protozoan infecting dogs and herbivores; it causes abortions in female herbivores and acquired neosporosis in dogs. Little information is available on these parasites in Tunisia.

The authors present herein, molecular studies of these three protozoa infection prevalences in Tunisian Beef, lamb and goat meats. PCR tests were performed in meat samples from Northern Tunisian.
Toxoplasma gondii molecular infection prevalence varied in sheep between 12.7 and 33.3%; it was estimated to 32.5% in goats and 19.3% in cattle.
The infection prevalence of goats by *Neospora caninum* was estimated to 19% (the prevalence in sheep and cattle will be available in few weeks).
The molecular prevalences of *Sarcocystis* spp. in sheep and goats were 58.6% and 50.4%, respectively. The overall infection prevalence of Sarcocystis spp. was 38%. Two species were identified, namely *S. hominis* (25%) and *S. cruzi* (12%).
There are few studies on these three protozoa in Tunisian ruminants; further investigations are needed to improve our knowledge on the epidemiological situation of toxoplasmosis, sarcosporidiosis and neosporosis and suggest suitable control measures.

VPA-P-11
Tropical theileriosis in North Africa: from Kouba vaccine to recombinant vaccines

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Tropical theileriosis (*Theileria annulata* infection) is very important cattle tick-borne disease in North Africa. In North Africa, this disease seems to have different epidemiological patterns with four vector tick species: *Hyalomma dromedarii*, *H. scupense*, *H. lusitanicum* and probably *H. anatolicum*.
During the early 20 ies Sergent, Donatien and Lestoquard developed a live vaccine (Kouba strain) which was intensively injected to thousands of cattle in Morocco, Algeria and Tunisia.
Cell culture vaccines, based in local strains, were produced in Morocco and Tunisia but they were not used at large scale the field. These vaccines conferred an excellent protection against the disease but not against the infection. The animals remain carriers; they play a reservoir role for ticks and show a decrease in milk yield. Moreover, these vaccines show low efficacy against heterologous strains.
Recombinant vaccines were used to immunize cattle against *T. annulata* infection in Tunisia. Several antigens were used with the prime-boost approach and showed that the best protection was obtained with SPAG-1 antigen associated to adjuvant (RWL). Indeed, 3 out of 6 animals survived (12 control animals died). The immunised cattle showed a significant increase in both pre-patent and the incubations periods. The association of SPAG-1 antigen associated to cell culture showed a synergy.
The perspective of recombinant vaccines seems to an excellent solution to control tropical theileriosis infection in North Africa. Indeed, this type of vaccines are safe, they are not transmitted to ticks and could be used to prevent different strains of parasites.
Further developments of vaccines are needed in order to control this very important parasitic disease.

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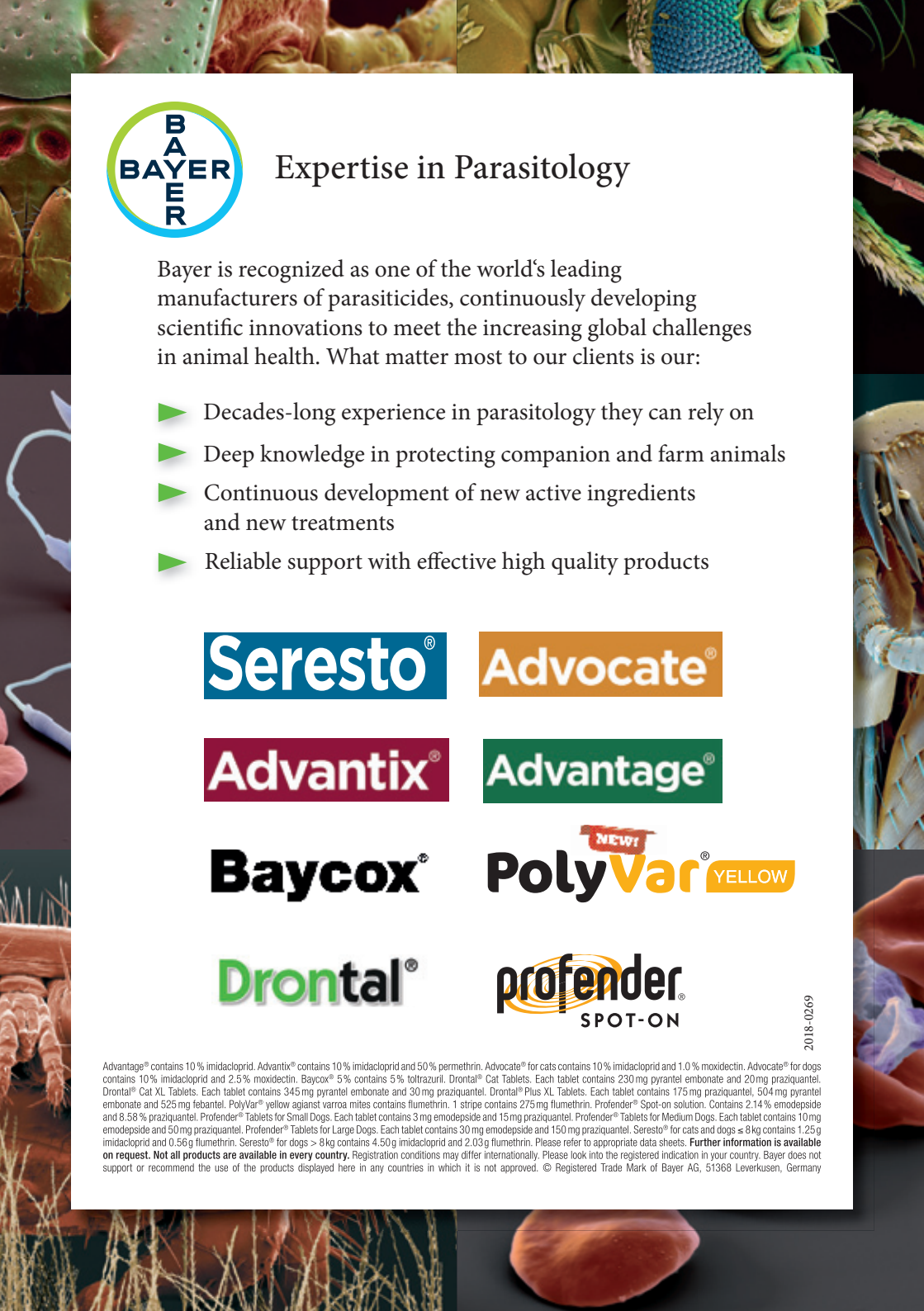

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