



19-22 June 2019
Quimbaya, Quindío, Colombia
Decameron Panaca

15th biennial meeting on *Toxoplasma* Biology and Toxoplasmosis

PRESIDENT OF THE CONGRESS:

Jorge Gomez-Marin, Universidad del Quindio

SCIENTIFIC COMMITTEE

Alejandra de-la-Torre, Universidad del Rosario
Karen Shapiro, University of California - Davis
Jeroen Saeij, University of California - Davis
Sebastian Lourido, Whitehead institute
David Roos, University of Pennsylvania

ORGANISING COMMITTEE

Sonia Guerrero - Manager
Margoth Coba - Administrative Agent
German Lamoroux - Web master
Registration and payment platform: Asistiendo, Antorami

SESSIONS

	Discussion leaders
Session I Epidemiology and environmental studies	Patricia Conrad- David Fergusson
Session II Immunology I	Jonathan Howard- Eva Frickel
Session III Cell cycle	Lilach Sheiner- Maria Francia
Session IV Biochemistry I	Maryse Lebrun - Sebastian Lourido
Session V Genomics, transcriptomics and evolution	David Roos- Jessica Kissinger
Session VI Host parasite interactions I	Martin Blume- Sebastien Besteiro
Session VII Immunology II	Nestor Cardona- Kirk Jensen
Session VIII Clinical toxoplasmosis	Alejandra de la Torre - Alexander Pfaff
Session IX Trafficking pathways	Moritz Treeck- Vern Carruthers
Session X Host parasite interactions II	Aylan Arenas- Jeroen Saeij
Session XI Biochemistry II	Lena Pernas-John Boothroyd

Process of selection of abstracts: All abstracts were reviewed by two blinded evaluators



Awards

FIRST, SECOND AND THIRD PRIZES

(Sponsored by Frontiers in Cellular Infection and Microbiology for Best Oral Presentations from PhD students or Postdoc)



BEST ORAL PRESENTATION IN THE FIELD OF GENOMICS

(Journal of Microbial Genomics of the Society of Microbiology)



ASM BEST POSTER PRIZE FOR PHD STUDENT AND ASM BEST POSTER PRIZE FOR LATIN-AMERICAN PHD STUDENT -WORK MADE IN LATIN AMERICA-

(Sponsored by ASM)



Environmental Toxoplasmosis Workshop

17-18th June 2019, University of Quindío
Auditorium Euclides Jaramillo Arango

JUNE 17th: SCIENTIFIC PRESENTATIONS

Workshop topic	Speakers	Hours
XVth International Toxoplasma Congress Official Opening	President of the Universidad del Quindío, Ing. Jose Fernando Echeverry Vicepresident of Research, Universidad del Quindío, Jorge E. Gomez Marin	8:00 – 9:00 a.m
Workshop Introduction	Chairs: Aurélien Dumètre & Karen Shapiro	9:00 – 9:10 a.m
Biology and metabolism of oocysts	David Ferguson	9:10 – 10:30 a.m
Dynamics of felid oocyst shedding	Elizabeth VanWormer	9:50 – 10:30 a.m
Coffee 10:30 - 11:00 a.m		
Environmental transmission	Karen Shapiro	11:00 – 11:40 a.m
Methods of oocyst inactivation in environmental samples	Aurélien Dumètre	11:40 – 12:20 a.m
Tools for the detection and persistence of oocysts in environmental samples	Isabelle Villena	12:20 – 1:00 p.m
Lunch 1:00 - 2:30 p.m		
<i>Toxoplasma</i> transmission perspectives from Brazil	Lilian Bahia-Oliviera	2:30 – 3:00 p.m
<i>Toxoplasma</i> transmission perspectives from Canada	Pia Muchaal	3:00 – 3:30 p.m
Coffee 3:30 - 4:00 p.m		
NIH Funding opportunities for Parasitology international research	Glen McGugan	4:00 - 4:30 p.m
Round table Part 1: Synergies and missing links in oocyst research and environmental transmission	Moderators: Aurélien Dumètre & Karen Shapiro	4:30 - 5:30 p.m
Dinner. All participants. 6:30 p.m		

JUNE 18th: FIELD EXPEDITION AND DISCUSSION

Workshop activity	Organizing persons	Hours
Field trip: forest reservation “La Montaña”	José Anibal Gonzalez and Fabiana Lora	9:00 a.m – 4:00 p.m
Coffee 4:00 - 4:30 p.m		
Round table Part 2: Synergies and missing links in oocyst research and environmental transmission	Moderators: Aurélien Dumètre, Isabelle Villena, Patricia Conrad, Fabiana Lora & Karen Shapiro	4:30 – 5:30 p.m

Precongress “EuPath DB workshop”

(Curso de Bioinformática avanzada: Manejo de bases de datos biológicos)
Maestría y Doctorado en Ciencias Biomédicas
Facultad de Ciencias de la Salud- Universidad del Quindío

Professors

David Roos; E. Otis Kendall Professor of Biology; University of Pennsylvania
Omar Harb; Director of Scientific Outreach and Education; University of Pennsylvania
Sebastian Lourido; Assistant Professor; White Head Institute
Jeroen Saeij; Associate Professor; UC Davis
Catalina Alvarez; Instituto Gulbenkian de Ciência

MONDAY 17th JUNE

8:00 - 9:00 a.m	Opening XV th <i>Toxoplasma</i> International Congress (Theater Euclides Jaramillo)
9:00 - 9:30 a.m	Workshop logistics & Introduction to ToxoDB
9:30 - 11:00 a.m	Finding genes and basic search strategies
11:00 - 12:00 p.m	Genome Browser I: Introduction to the genome browser
12:00 - 1:00 p.m	Lunch
1:00 - 3:00 p.m	Genome Browser II: Interpreting RNAseq data
3:00 - 3:30 p.m	Coffee break
3:30 - 4:30 p.m	Orthology and Phylogenetic Profiles
4:30 - 6:00 p.m	RNA sequence data analysis via Galaxy, Part I Uploading data and starting the workflow (Group Exercise)

TUESDAY 18th JUNE

8:00 - 9:00 a.m	Sequence Exercises: Motifs, domains and colocation
9:00 - 12:00 p.m	RNA sequence data analysis Part II: viewing and analyzing your results (Group Exercise)
12:00 - 1:00 p.m	Lunch
1:00 - 3:00 p.m	Functional Genomics I: Transcriptomics, Proteomics, GO Enrichment, Metabolic Pathways
3:00 - 3:30 p.m	Coffee break
3:30 - 4:30 p.m	Functional Genomics II: Host Response datasets
4:30 - 6:00 p.m	Population Genetics

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In memoriam. Professor Ermanno Candolfi (1957-2019)

In memoriam. Professor Elmer Pfefferkorn (1931-2019) and

Dr. Lorraine Pfefferkorn (1937-2019)

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ORAL PRESENTATIONS

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International *Toxoplasma* Congress Toxo XV

ORAL PRESENTATIONS

WEDNESDAY 19 JUNE 2019

SESSION I - EPIDEMIOLOGY AND ENVIRONMENTAL STUDIES

101. Amazonian toxoplasmosis outbreak in an Amerindian village of French Guiana

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Abstract. Since recent decades, strains with particular phylogenetic profiles which differ from the clonal type circulating in North America and Europe (type I, II, III) have emerged especially in French Guiana. They are highly pathogenic and have defined a new clinical entity called Amazonian Toxoplasmosis occurring mainly in immunocompetent persons. We report one toxoplasmosis outbreak occurring in a french guianese remote forestian village on the Oya-pock River (Brazilian side). It has referred to 20 cases with confirmed serology in 9 households. Biological, epidemiological and environmental explorations permit to isolate two human strains and detect DNA *Toxoplasma* in the soil and the water collected around the cases 'households. The transmission mechanisms across the village are discussed as until now, the village was unscathed by AT outbreaks and seems to suddenly fulfill the conditions for a transmission across the village (increase in the population of domestic cats, unsafe water sources.). Moreover, this outbreak of symptomatic AT calls for increased awareness of practitioners in this area.

Funding. We want to thank "le departement des Centres Délocalisés de Prévention et de Soins", Hôpital Andrée Rosemon, Cayenne, French Guiana. They contribute to the logistic support for the exploration of the outbreak.

102. *Toxoplasma gondii* in cats in Denmark: seroprevalence and risk factors

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Abstract. Despite domestic cats are important hosts for the zoonotic parasite *Toxoplasma gondii*, information about feline *T. gondii* infections has been lacking in Denmark. In this study, we estimated *T. gondii* seroprevalence among cats visiting five veterinary clinics in Copenhagen, Denmark. Samples from 139 pet cats, collected from September 2018 to January 2019, were tested for antibodies against *T. gondii* using a commercial indirect ELISA. The apparent seroprevalence was 21% (95% confidence interval: 15-28); exposure to *T. gondii* was common in the investigated pet cat population. Seropositivity was positively associated with some lifestyle-factors, in particular with having outdoor access and with diet including raw meat. Seropositive cats have presumably shed oocysts of the parasite after acquiring the infection; after sporulation, these oocysts can serve as infection source to other hosts. It was noteworthy that 40% of the cats had outdoor access and 18% of the cat owners reported flushing their cats' faeces down the toilet – both activities allow oocysts, if present in the faeces, to end up in the environment. Based on the results of this study, preventing feline *T. gondii* infections and preventing environmental contamination with oocysts should merit more attention in Denmark. This poster was also presented at DSP Spring Symposium.

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103. Multi-Scale Model of *Toxoplasma* transmission

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Abstract. The intracellular parasite *Toxoplasma gondii* continues to exist thanks to a concert of phenomena that occurs at multiple scales. In this study, we address the theoretical aspects of studying *T. gondii* transmission as a chain of correlated events that start at the molecular level via mechanisms of cell invasion, and are continuously connected to: immune function, domestic and wildlife host movement, food networks, land tenure and use, climate, social interactions, and individual/communal knowledge, attitudes and practices. All these linked analyses provide a comprehensive picture that no single scale can produce in isolation. The usefulness of models under this light takes on new meanings, and this broad scope requires the cooperation of scientists coming from very different intellectual traditions. This study will present a global framework that will demonstrate the contribution of each scale for disease transmission, providing insights into *T. gondii* management in an endemic system.

Funding. University of Georgia and Universidad del Quindío.

104. Seroprevalence and risk factors of *Toxoplasma gondii* in sheep and Goats in French Guiana

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Abstract. Background: *Toxoplasma gondii* is a zoonotic protozoan parasite and it infects warm-blooded animals, including humans, livestock, and marine mammals. By being highly susceptible to *T. gondii* parasite, sheep and goats play an important role in the human transmission. We evaluate the seroprevalence and risk factors of *T. gondii* infection in small ruminants in French Guiana. Methods: Blood samples were collected from 507 smalls ruminants (229 sheep and 278 goats) between January 2015 and December 2017 corresponding to 17 farms. Sera from animals were tested for antibodies to *T. gondii* by the modified agglutination test (MAT). Epidemiological data were collected through a comprehensive questionnaire. Statistical analysis was used to identify a possible association between several identified risk factors. Results: Specific antibodies to *T. gondii* were found respectively in 180 and 200 of the 229 sheep and the 278 goats tested (78.6%; 95%CI: 73.3-83.9; 71.9%; 95%CI: 66.6-77.2). The seroprevalence was higher in sheep as compared to goats. We then identified four variables potentially associated with toxoplasmosis infection on the farms ($p < 0,05$), such as the age of the animal, the presence of cats on the farm, a stream and well water consumption Conclusion: This study is a first report of toxoplasmosis seroprevalence among the livestock animals in French Guiana. The very high seroprevalence demonstrates that more attention has to be given to the sheep and goat meat and farm activities. Moreover, the results suggest a highly environmental contamination from soil and water that should be biologically confirmed.

Funding. We thank the Labex Ceba (Center for study of the amazonian biodiversity) that financially contributed to the study.

105. Distribution and virulence of *Toxoplasma gondii* genotypes in southern sea otters (*Enhydra lutris nereis*)

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Abstract. The observation that only some *Toxoplasma gondii*-infected southern sea otters (*Enhydra lutris nereis*) develop fatal toxoplasmosis, while others have incidental or mild infections, has long puzzled parasitologists. This study combined molecular characterization of *T. gondii* isolates from southern sea otters with results from detailed postmortem examinations, providing a unique opportunity to evaluate whether parasite genotype influences pathological outcome. Molecular findings were further combined with data on *T. gondii* genotypes circulating in sympatric terrestrial carnivores to examine spatial associations among parasite genotypes on land and sea. Genotyping was performed on 135 *T. gondii* isolates obtained from sea otter brain tissue; 116 of these same animals also received comprehensive pathological examination. In all cases, assessment of *T. gondii*-associated lesion patterns and disease outcome was performed by a pathologist with no knowledge of the *T. gondii* genotypes obtained from brain tissue through isolation in cell culture. The predominant *T. gondii* genotypes identified were Types X, atypical mixed II/X, and X variants. These atypical genotypes accounted for 79% of *T. gondii* isolates, with the remainder belonging to Type II. Type X or mixed X variants were the only genotypes isolated from the brains of otters that died from *T. gondii* as a primary cause of death. Spatial analysis revealed identical *T. gondii* genotypes circulating in terrestrial carnivores and sea otters in central California. Results demonstrate a land-sea connection of virulent *T. gondii* genotypes and highlight the need for long-term interdisciplinary research for unraveling mechanisms of *T. gondii*-induced morbidity and mortality in wildlife populations.

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partment of Fish and Wildlife (CDFW), especially Francesca Batac and Laird Henkel, for their assistance with project logistics. We also thank volunteers and staff at CDFW, The Monterey Bay Aquarium, The Marine Mammal Center, and other stranding agencies for their efforts to recover sick and dead-stranded sea otters along the central California Coast. Brittany Dalley, Lezlie Rueda, and Mitchell Ng, are acknowledged for technical assistance with molecular characterization assays. Financial support for this work was provided by the AAZV Wild Animal Health Fund.

106. Food safety assessment and risk for foodborne toxoplasmosis in school restaurants in Armenia; Colombia

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Abstract. We assessed the risk for foodborne toxoplasmosis in 10 school restaurants in Armenia (Quindío, Colombia). We analyzed the presence of *Toxoplasma* DNA in the food, water, and living and inert surfaces of school restaurants, and we correlated these findings with the results of food safety inspection scores and with the prevalence of specific anti-*Toxoplasma* antibodies in children who ate at these restaurants. We found 13/213 (6.1%) of the samples were positive using PCR to test for *T. gondii* DNA. Water samples had a significantly greater frequency of PCR positive results (17% in water vs. 3.5% for the rest of the samples, $p = 0.004$). In 60% (6/10) of the public-school restaurants, there was at least one PCR *Toxoplasma*-positive sample. In 311 serum samples from children who attended the restaurants, 101 (33%) were positive for IgG and 12 (3.9%) for IgM anti-*Toxoplasma*. The median of the compound score for the fulfillment of inspection for food safety conditions was of 60.7% (range 50-72). Higher *Toxoplasma* PCR positivity in surfaces, food, or water at each restaurant was correlated with lower inspection scores for water supply and water store conditions. Lower scores in disinfection procedures were correlated with a higher prevalence of IgG and IgM antibodies in children who ate at those restaurants. Inspection scores can identify restaurants with a higher risk for the presence of *Toxoplasma*.

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107. *Toxoplasma gondii* seroprevalence in pregnant women from a rural population of Buenos Aires province; Argentina

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Abstract. *Toxoplasma gondii*, the causative agent of toxoplasmosis, is an obligate intracellular parasite that infects a wide variety of warm-blooded animals, including man. Infection with *T. gondii* is very common in humans throughout the world, being the intake of raw or undercooked meat, fruits, vegetables and water contaminated with parasite cysts the main entry routes. Toxoplasmic infection has been associated with rural regions as well. Here, we analyzed the prevalence of anti-*T. gondii* antibodies in Chascomús, a city immersed in a rural area, detecting a 34.5% of seropositives. The seroprevalence and different habits were analyzed: Activity (urban and rural), home water supply, animal husbandry, presence of cats as pets, gardening and consumption of meat and their frequencies (pork, sheep and sausages), not significant differences were found for those variables. Significant differences were only found when the seroprevalence was analyzed between the urban and suburban regions of the city of Chascomús, being higher in the latter. Given that the suburban area is not yet fully populated, with several empty lots and lack of some essential services, it is presumed that the higher seroprevalence could be due to an unfavorable socioeconomic situation and/or a higher possibility of predation of domestic cats to infected rodents.

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THURSDAY 20 JUNE 2019

SESSION II - IMMUNOLOGY

108. Role of Immunity-Related GTPases for maintaining virulent *Toxoplasma gondii* in wild rodents

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Abstract. 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 prey more on other rodent species, e.g. *Myodes glareolus*, *Microtus* spp. and *Apodemus* spp. They also show higher *T. gondii* seroprevalences and less susceptibility to virulent strains compared to *Mus* spp., implying they could be more relevant intermediate hosts. We aim at assessing whether specific IRGb2-b1-like proteins confer resistance to virulent *T. gondii* infection in these species, similar to *Mus* spp. Established *M. glareolus* cultures of Bone Marrow-Derived Macrophages, primary fibroblasts and a kidney cell line, show evidence of control of virulent parasite infection upon treatment with a custom-produced recombinant vole IFN- γ . Irg-like sequence of *M. glareolus*, *Microtus* spp. and *Apodemus* spp. in Germany show amino acid diversity between and also within these species, similarly to wild-derived *M. musculus*. *M. musculus* castaneus CIM strain resists infection with virulent parasites, which eventually form brain cysts. To investigate whether resistance is mediated by polymorphic IRGb2-b1-like proteins, we knocked-out the resistant Irgb2-b1 gene from a CIM cell line and introduced a *M. glareolus* Irgb2-b1-like sequence. Results on the virulence phenotype will be presented and will help to assess the ecological importance of *M. glareolus* as intermediate hosts for virulent parasite transmission to cats.

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109. Impact of *Toxoplasma gondii* ropphy proteins ROP5 and ROP18 on inflammasome activation and cell death

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Abstract. Cell-autonomous immune response against *T. gondii* in mice is largely dependent on two families of IFN- γ -inducible proteins, the Immunity-Related GTPases (IRG proteins) and Guanylate Binding Proteins (GBP proteins). Accumulation of these proteins at the parasitophorous vacuole membrane is required for parasite control and subsequent cell death. Several polymorphic *T. gondii* effectors were identified to directly inactivate IRG proteins resulting in maintenance of PVM integrity. The same parasite effectors interfere with GBP recruitment to the PVM. In contrast to IRG protein inactivation, detailed functional analysis of *T. gondii* virulence effectors in context of GBP protein inactivation is not yet available. In recent studies it was shown that GBP5 promotes selective NLRP3 inflammasome responses to pathogenic bacteria. We have found a direct interaction of ROP5 and ROP18 with mGBP5 and aim to elucidate a potential functional impact of this interaction in terms of inflammasome activation upon *T. gondii* infection. We determined different inflammasome markers and cell death in mouse bone marrow-derived macrophages (mBMDM) and dendritic cells (mBMDC) infected with *T. gondii*. Higher levels of IL-1 β secretion were observed in RH Δ rop5 infections compared to RH or RH Δ rop18. Using specific knockout mBMDCs cells we verified that this IL-1 β

release is dependent on NLRP3, ASC and Caspase 1, but independent of Gasdermin-D. Differences in IL-1 β secretion do not correlate with cell death. We were able to show that cell death is only related to vacuolar disruption upon infection with different *T. gondii* strains suggesting an additional role of ROP5 at the inflammasome level.

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110. Molecular basis of *Toxoplasma* PV membrane targeting by IRGB6

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Abstract. Gamma interferon (IFN- γ) stimulates expression of IFN-inducible GTPases such as p47 immunity-related GTPases (IRGs) and p65 guanylate binding proteins (GBPs) in mice. Both GTPases are shown to accumulate *Toxoplasma gondii* parasitophorous vacuole membranes (PVMs), facilitating the parasite clearance. Recruitment of IRGs and GBPs to *T. gondii* PVM are mutually regulated, however, the molecular mechanisms of how these GTPases are loaded on the membrane to mediate the eventual damage are poorly understood. Here we analyzed the role of an IRG member IRGB6 in *T. gondii* PVM recruitment and damage. IRGB6-deficient mice were highly susceptible to the parasite challenge *in vivo*. IRGB6-deficient cells showed no signs of the PVM damage and demonstrate severely defective recruitment of IRGA6 and GBPs to the PVM, accounting for the super susceptibility *in vivo*. Series of biochemical analysis revealed that not only the GTPase activity in the N-terminal GTPase domain but also lipid binding moiety in the C-terminus are essential for the PVM targeting. Moreover, recombinant IRGB6 caused strong membrane tubulation on liposomes containing defined lipids. Taken together, our results suggest that IRGB6 lipid binding and GTPase activities are pivotal for the PVM destruction, leading to subsequent loading other IRGs or GBPs and efficient host defense *in vivo*.

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111. Human GBP1 drives apoptosis of *Toxoplasma*-infected macrophages

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Abstract. The guanylate-binding protein (GBP) family of interferon-inducible GTPases promote antimicrobial immunity and pyroptotic cell death. Whether GBPs regulate other forms of cell death is not known. The apicomplexan parasite *Toxoplasma gondii* causes human macrophage death through unidentified mechanisms. Here we report that *Toxoplasma*-induced death of human macrophages required GBP1, and its ability to target *Toxoplasma* parasitophorous vacuoles through its GTPase activity and prenylation. Mechanistically, GBP1 promoted *Toxoplasma* detection by AIM2, which induced GSDMD-independent, ASC- and caspase-8-dependent apoptosis. Notably, GBP1 could be bypassed by the delivery of *Toxoplasma* DNA into the cytosol, pointing to its role in liberating microbial molecules. GBP1 acts as a gatekeeper of cell-death pathways, which respond specifically to infecting microbes. Our findings expand the immune roles of human GBPs in regulating not only pyroptosis,

but also apoptosis.

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112. Role of IFN- γ -dependent ubiquitination in the resistance of mouse cells against *T. gondii*

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Abstract. In mice, IFN γ -induced immunity-related GTPases (IRGs) target the parasitophorous vacuole membrane (PVM), inducing its rupture, death of the parasite and necrotic death of the host cell. Following IRG recruitment, guanylate binding proteins (GBPs), ubiquitin (Ub) and autophagy (Atg) components are also recruited to some PVs but the role of downstream recruitment events in resistance is rather unclear. Approximately 50% of vacuoles of avirulent strains of *T. gondii* are ubiquitinated in IFN γ -primed mouse cells. This Ub coat colocalizes to some extent with IRG proteins. It has been suggested, but not shown, that IRG proteins may be the primary target of ubiquitination. Our results suggest that PVM ubiquitination is dependent on prior loading of IRG proteins but independent of the presence of any one specific IRG protein. IRG proteins themselves are probably not the target of ubiquitination, and most of the ubiquitination observed is at the surface of the parasite itself, subsequent to IRG-mediated damage to the PVM. Two E3 ubiquitin ligases implicated in resistance against *T. gondii* are fully dispensable for IRG protein-mediated cell-autonomous resistance in vitro. When virulent *T. gondii* strains infect cells from susceptible mouse strains there is little or no vacuolar disruption or ubiquitination, associated with deficient IRG protein loading. However cells from the fully resistant mouse strain, CIM, which load IRG proteins, also ubiquitinate vacuoles from virulent *T. gondii* strains.

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113. Regulation of IL-1 β Production and Release During *T. gondii* Infection of Primary Human Monocytes

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Abstract. Monocytes are rapidly recruited to sites of *T. gondii* infection and contribute to host defense by initiating a robust inflammatory response partially mediated by IL-1 β release. *T. gondii* induces activation of the NLRP3 inflammasome for the processing and release of IL-1 β , but little is known about the mechanism of activation of the NLRP3 inflammasome in primary human monocytes during infection with live and complex pathogens. We find that *T. gondii* infection induced spleen tyrosine kinase (Syk) phosphorylation, and Syk inhibition reduced IL-1 β release from *T. gondii*-infected, primary human monocytes. The Syk inhibitor R406, and inhibitors of PKC δ , CARD9/MALT-1 and IKK all decreased parasite-induced IL-1 β transcripts, pro-IL-1 β production, IL-1 β release and phosphorylation of the NF- κ B subunit p65, indicating that Syk likely functions upstream of this NF- κ B-dependent signaling pathway to induce IL-1 β transcriptional activation during *T. gondii*-infection. While, IL-1 β is thought to be released primarily through an inflammatory form of cell death called pyroptosis, which is driven by Gasdermin-D cleavage, viability assays indicated that *T. gondii*-infected monocytes released IL-1 β but did not undergo

pyroptosis, did not become PI+ and Gasdermin-D remained uncleaved. Taken together, these data indicate that *T. gondii* induces a Syk-NLRP3-caspase-1 pathway of inflammasome activation and IL-1 β release in primary human monocytes, which does not involve Gasdermin-D cleavage or pyroptosis.

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114. Highly prevalent Irgb2-b1PWK allele in South American mice provides advantageous resistance against local *Toxoplasma gondii* strains

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Abstract. Host-parasite interactions drive co-evolutionary processes in nature. For *Toxoplasma gondii*, coadaptation with its intermediate hosts demands enough virulence to survive the host's immune attack and encyst in the brain, but not so much that the host dies of the infection. Immunity-related GTPases (IRGs) are IFN γ -inducible genes of an essential cell-autonomous resistance system in the house mouse, *Mus musculus*, that is complicit in establishing this delicate balance. Paradoxically, certain virulent strains of the parasite inactivate IRG effector proteins and thus kill mice and therefore also themselves, within a few days. Some mice, however, express specific alleles of a distinctive IRG gene, Irgb2-b1, that act as antagonists to virulence and restore the co-adaptive balance. The molecular basis of virulence and its reversal by Irgb2-b1 are now largely understood. Interaction between South American (SA) *T. gondii* strains and Eurasian mice is only 500 years old and virtually all SA *T. gondii* tested are virulent for laboratory mice and some Eurasian wild-derived strains. However, interactions between SA mice and *T. gondii* strains are still uncharacterized. We have found that Brazilian *M. m. domesticus* have an exceptionally high prevalence of the Eurasian Irgb2-b1PWK allele, which is unlikely due to a founder effect. In vitro experiments in mouse cells that are carriers of the Irgb2-b1PWK allele, showed efficient control of local SA *T. gondii* strains. The prevalent Irgb2-b1PWK allele has therefore probably been selected from the IRG allele pool imported by Eurasian mouse immigrants because it provides resistance against local SA *T. gondii* strains.

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SESSION III - CELL CYCLE

115. The *Toxoplasma gondii* cyst wall: Composition and Interactome

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Abstract. The tissue cyst of *T. gondii*, found in latent infection, serves a critical role in both transmission and reactivation of this organism. While the cyst wall is clearly delineated by ultrastructural analysis, the function of this layer in host/parasite interactions is not fully understood and the full protein composition of this structure is unknown. A method was developed to purify cyst wall fragments (utilizing percoll gradients and CST1 immune-magnetic beads), and a proteomic analysis of purified cyst wall fragments was performed. Known cyst wall proteins, such as CST1, BPK1, MCP4, MAG1, GRA2, GRA3, and GRA5, were identified in this preparation by LC-MS/MS. In addition, dense granule proteins (GRAs) not previously shown to associate with the cyst wall, as well as uncharacterized hypothetical proteins were also identified. Several of these hypothetical proteins were epitope tagged, and IFA confirmed these proteins as novel cyst wall/matrix proteins (CST proteins). To further understand how these and other proteins interact, several cyst wall proteins (CST1, BPK1, MAG1, GRA6, and MCP4) were tagged with a promiscuous biotin ligase (BirA*) and their interacting partners were analyzed. Within this interactome, previously

described cyst wall proteins (including GRAs), as well as several uncharacterized hypothetical proteins were identified. These hypothetical proteins were validated as new protein components of the cyst wall, deleted to assess cyst biology, and epitope tagged with BirA* to identify their interacting proteins, producing an extensive cyst wall interactome. These studies have provided the groundwork to understand cyst wall formation and insights into the biology of latency.

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116. Loss of a bradyzoite-specific cyclin leads to a “super tachyzoite” that is more virulent and completely resistant to alkaline-stress.

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Abstract. *Toxoplasma gondii* infections continue to be a public health hazard for millions of individuals that contract this pathogen annually. Individuals can be treated (despite significant side effects) for acute clinical toxoplasmosis, however, no current drug successfully treats or prevents the tissue cyst that is responsible for long-term infections. The *Toxoplasma* biology that underlies the establishment of a chronic infection is the developmental conversion of the acute tachyzoite stage into the latent bradyzoite stage. The molecular basis of chronic infection is not well understood, although experimental evidence demonstrates this transformation is accompanied by significant changes in gene expression. Among the changes associated with bradyzoite development is the expression of a bradyzoite-specific cyclin (called P3-Cyc), which is related to the PHO80 cyclin family of yeast. We have determined P3-Cyc is cytoplasmic and highly expressed in parasites exposed to alkaline-stress, while nearly undetectable in tachyzoites. By contrast, developmentally competent tachyzoites express a related P-cyclin, P2-Cyc, which is exclusively localized to the parasite nucleus. Disrupting P3-Cyc expression using direct or auxin-conditional knockout approaches, strongly inhibits in vitro differentiation of the tachyzoite into the bradyzoite. Unexpectedly, Me49 tachyzoites lacking P3-Cyc become “super parasites” capable of full survival and rapid growth in alkaline-stress conditions, which is reversed by P3-Cyc expression. Consistent with these changes, the absence of P3-Cyc also increases parasite virulence in mice. Experiments are underway to understand P3-Cyc interaction with other parasite proteins as well as the effect of P3-Cyc on developmental gene expression.

Funding. This work is supported by NIH-NIAID.

117. Regulation of *T. gondii* cell-cycle dependent expression profiles by ApiAP2 transcription factors

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Abstract. Gene expression is tightly regulated during the tachyzoite cell cycle. Although the timely expression of number of cell-cycle regulated genes is crucial for parasite division, the molecular mechanisms controlling their regulation remain poorly understood. ApiAP2 are a family of conserved transcription factors (TF) that play an important role in regulating gene expression in apicomplexan parasites. ApiAP2 proteins that may control the cell cycle dependent expression program are to be discovered. To better understand how these cell-cycle dependent gene expression profiles are established, we identified ApiAP2 proteins showing a cell cycle dependent expression. Using the Auxin-degradation system, we produced several inducible knock-down (iKD) mutant for these ApiAP2 proteins. Among them, a cell-cycle regulated ApiAP2 is expressed during the late S-phase of the tachyzoite cell-cycle. The iKD mutant parasites for this gene are unable to proliferate. In presence of Auxin, the mutant parasites do not produce daughter cells and are arrested in the early phase of budding. This indicates that this TF may specifically regulate the early steps of the daughter cell formation. Using RNA-Seq, we demonstrate that the level of expression of number of transcripts is affected by the knock-down of this potential TF. These regulated genes may represent crucial factors controlling the budding cycle in this parasite. To better understand the molecular mechanisms at stake, we also investigated the binding ability of this TF by ChIP-seq. This is the first evidence of the control by an ApiAP2 TF of a specific step of the tachyzoite cell-cycle.

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118. Study of the biological importance of *Toxoplasma gondii* H2B.Z histone and function of its acetylated lysines

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Abstract. *T. gondii* and other apicomplexan parasites possess an unusual H2B variant called H2B.Z which is found in nucleosomes containing H2A.Z, another histone variant. The existence of a unique H2B variant in *T. gondii* offers an interesting matter of study to find new biological pathways and putative drug targets. H2A.Z and H2B.Z are positioned at transcription start sites (TSS) together with H3K4Me3 and both histone variants are highly acetylated at their N-terminal tails. Mutagenesis studies were performed to determine the biological importance of H2B.Z, particularly the role of the N-terminal lysines. We over-expressed myc-tagged H2B.Z in Pru strain in which the acetylable lysine residues were maintained (c-Myc-WT) or mutated to either the neutral amino acid alanine (c-Myc-A) or the positively charged arginine (c-Myc-R). The resulting transgenic parasites did not show significant differences in replication rate, but we observe changes in the rate of bradyzoite differentiation. Parasites ectopically expressing c-Myc-R/H2B.Z differentiated more compared with parental, while c-Myc-A/H2B.Z had a lower differentiation rate (1.88 and 0.74, respectively, while c-Myc-wt/H2B.Z rate was 1.23 relative to parental taken as 1). Since the H2B.Z gene is essential, we generated new mutants by disruption of the endogenous H2B.Z gene using CRISPR/Cas9 in the lines over-expressing the mutant histones (c-Myc-A/KO and c-Myc-R/KO). Phenotypic analysis showed similar results to those obtained with the over-expressing lines: there is a higher differentiation rate in c-Myc-R/KO parasites, and lower in c-Myc-A/KO. These findings suggest that a correct acetylation of the N-terminal region of H2B.Z is relevant for the control of parasite development.

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119. A photoactivatable crosslinking system reveals organization of the *Toxoplasma* inner membrane complex

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Abstract. The *Toxoplasma* inner membrane complex (IMC) is an important organelle involved in parasite motility and replication. The IMC resides underneath the plasma membrane and is composed of flattened membrane vesicles supported by a rigid cytoskeletal network. Although the protein composition of the IMC is becoming better understood, the protein-protein associations that enable proper functioning of the organelle remain largely unknown. Determining binding partners in the IMC cytoskeletal network is particularly challenging, as solubilizing the cytoskeleton requires harsh conditions that often disrupt protein complexes. To circumvent this problem, we demonstrate the application of a photoreactive unnatural amino acid (UAA) crosslinking system to capture protein interactions in their native environment. In addition to identifying partners, the UAA approach maps the binding interface of the bait protein used for crosslinking, providing structural information of the interacting proteins. We applied this technology to the essential IMC protein ILP1 and demonstrate that distinct regions of the C-terminal coiled-coil domain of ILP1 crosslink to the alveolins IMC3 and IMC6, as well as IMC27. We also show that the IMC3 C-terminal domain and the IMC6 N-terminal domain are necessary for crosslinking to ILP1, further mapping interactions between ILP1 and the cytoskeleton. Together, UAA crosslinking provides a new approach to study protein-protein interactions in *Toxoplasma* and reveals new insight into the architecture of the cytoskeletal network of the apicomplexan IMC.

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120. Pushing the Envelope: Unconventional Kinetochores and Modes of Chromosome Segregation in parasitic Alveolates

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Eukaryotic cellular life is wondrously diverse. How such diversity emerges while core systems are maintained is a major question for specialized parasitic lineages. An example of a divergent but essential process is chromosome segregation. At the heart of this operation are kinetochores: protein assemblies that connect chromosomes to spindle microtubules during mitosis. Although human and yeast kinetochores are largely similar, comparative genomics analyses indicate extensive alterations of kinetochores in diverse eukaryotes. The eukaryotic superphylum Alveolata harbours the seemingly disparate lineages of dinoflagellate algae and apicomplexan parasites, which both have non-canonical mechanisms of chromosome segregation and show degenerated kinetochore composition. Dinoflagellate kinetochores are embedded in the nuclear envelope so that extra-nuclear spindle microtubules, which run through channels of the nucleus, can reach them and segregate chromosomes. Apicomplexans have a specific nuclear compartment called the centrosome that harbours the spindle apparatus and connects the centrosome and kinetochores on opposite sides of the nuclear envelope throughout the cell cycle. To understand how these kinetochores evolved and function, we are aiming to make a parts list of kinetochores in Alveolates. We are setting up BioID on kinetochore-tagged *Toxoplasma gondii* and *Perkinsus marinus* strains and verify the location of (newly) identified kinetochore proteins by fluorescence microscopy. The extensive loss of ancestral kinetochore complexity, rapid sequence evolution, and the apparent high number of innovations, raise many questions about the evolution and function chromosome segregation in Alveolates. We would like to discuss our evolutionary cell biology approach for understanding aspects of *Toxoplasma gondii* cell biology.

121. Identification of a master regulator of differentiation in *Toxoplasma*

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Abstract. *Toxoplasma gondii* chronically infects approximately a quarter of the world's population. Recrudescence of latent infections can cause life-threatening disease in the immunocompromised and recurrent ocular lesions in the immunocompetent. Chronic infection is established when rapidly replicating tachyzoites differentiate into slow-growing bradyzoites, which form tissue cysts resistant to immune clearance and current therapeutics. Despite its central role in the *Toxoplasma* life cycle, the molecular basis of differentiation is not understood. In this study, we provide an in-depth characterization of differentiation through stage-specific and single-cell transcriptional profiling. We functionally explore this transition using CRISPR-based screens to identify a putative transcription factor (BFD1) that is both necessary and sufficient for differentiation, providing a genetic handle for the investigation of chronic *Toxoplasma* infections.

SESSION IV - BIOCHEMISTRY I

122. Contribution of the apicoplast to *Toxoplasma* survival and persistence as a latent stage

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Abstract. Acute toxoplasmosis is associated with the rapid replication and spread of *Toxoplasma gondii* tachyzoites within the body. This infection phase is usually contained by the immune system in immunocompetent individuals. However, the parasites can differentiate into slowly growing bradyzoite forms, establishing within tissue cysts, primarily in the central nervous system and muscle. This persistent chronic form of the pathogen remains in the host throughout its life and can lead to a severe pathology in the event of a weakened immune system. *T. gondii* harbors an organelle called the apicoplast, derived from a secondary endosymbiotic event. This plastid contains several metabolic pathways, some of which are essential to tachyzoites. We are investigating the contribution of the organelle to the survival of chronic stage, which is currently incurable. We are using stage-specific promoters to generate conditional bradyzoites mutants of essential apicoplast genes. We are currently evaluating the impact of the loss of the organelle on the differentiation process of the parasites into the chronic latent form, as well as the survival of fully differentiated bradyzoites, both *in vivo* and *in vitro*. If we manage to generate apicoplast-deficient bradyzoites that are viable but fail to reactivate *in vivo*, this offers the possibility of using these avirulent parasites as potential vaccines, particularly in livestock to reduce zoonotic transmission. On the other hand, if the apicoplast is essential for the viability of bradyzoites, it would validate the organelle as a source for potential drug targets for this particular parasite stage.

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123. Contribution of Developmentally Regulated Metabolic Enzymes to Stage Conversion During *Toxoplasma* Pathogenesis

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Abstract. *Toxoplasma* is a ubiquitous intracellular protozoan parasite responsible for toxoplasmosis in humans. In infected immunocompromised individuals, a critical stage in the pathogenesis begins with an unknown stimulus triggering the conversion of its asexual forms from fast replicating tachyzoites to the slowly dividing bradyzoites. These forms of *Toxoplasma* have stage specific metabolic requirements that enable them to survive and be efficiently transmitted. Notably, electron micrographs revealed the presence of numerous starch granules (amylopectin) mostly found in the bradyzoites form. Being part of the metabolic pathways, very little is known about the role of amylopectin. Specifically, comparative transcriptomic analyses of bradyzoites and tachyzoites revealed that there exist many genes encoding metabolic enzymes with higher expression in chronic compared to its acute form. While enolase 1 and lactate dehydrogenase 2 are well-established players in bradyzoite biology, many genes remains poorly characterized. Very little is known about their contribution to tachyzoite-bradyzoite interconversion, cystogenesis, and initiation of infection in new host. After preliminary studies using ToxoDB, I have identified 2 genes glucose-phosphate mutase and glucose-6-phosphate isomerase to be highly upregulated, by at least 2-fold, in the chronic form. Targeting stage-specific genes involved in *Toxoplasma* differentiation will help uncover novel key players of *Toxoplasma* virulence. With many of these genes predicted to encode glycolytic enzymes, I hypothesize that mutations of these developmentally regulated enzymes will result in the dysregulation of amylopectin metabolism during *Toxoplasma* stage conversion *in vitro* and *in vivo*. Understanding the role of these enzymes will provide valuable insights in to *Toxoplasma* differentiation.

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124. Fat matters: global profiling of myristoylation in *Toxoplasma gondii* reveals an unexpected role for lipidation on a type-I microneme protein important for host cell invasion

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Abstract. N-myristoylation, the co-translational addition of myristate from myristoyl coenzyme A to the N-terminal glycine of proteins, is a ubiquitous type of protein lipidation. So far only a handful of myristoylated proteins have been discovered in *Toxoplasma* and include prominent examples such as TgCDPK3, TgPKG, TgGAP45 and TgARO, all implicated in crucial processes like parasite egress, motility and invasion. Here we describe the first global chemoproteomic screening of protein myristoylation in *Toxoplasma*. Through quantitative mass spectrometry-based proteomics coupled with novel chemoproteomic tools we identified over 70 known and novel myristoylated proteins and investigated two of them in more detail. Firstly we show that TgCDPK1 appears associated with parasite membrane structures distinct from TgCDPK3 and myristoylation is important but not essential for function. This has important implications for identifying targets and the function of TgCDPK1. Secondly, we identified myristoylation on a type-I transmembrane microneme protein (MIC7) thought to be bradyzoite specific. Using quantitative proteomics we show that mMIC is not stage-specifically regulated but present in tachyzoites and bradyzoites. We then establish a likely essential role for MIC7 in host cell attachment/ invasion through conditional gene deletion. By complementing the KO strain with wildtype or a myristoylation mutant we show that myristoylation is not important for trafficking of MIC7, but surprisingly, appears to play an important role in host-cell attachment/ invasion.

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125. Metabolomic perspective on the three major isotypes of *Toxoplasma gondii* and the formation of tissue cysts

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Abstract. The virulence of *Toxoplasma gondii* depends on distinct genetic factors during mice infections but is less well understood other animals and humans. However, it is ultimately dependent on the balance between pathogenic tachyzoites and clinically silent bradyzoites. The propensity to form dormant tissue cysts in vitro varies among different isolates and isotypes and is a result of the genotype and interaction with the host cell. We take an untargeted metabolomics perspective to understand how both factors influence the metabolome of this parasite and support stage differentiation. To this end we dissected the metabolic phenotype of six strains representative of the three isotypes using untargeted UHPLC-MS and GC-MS based metabolomics. Indeed, we find that self-organizing maps reveal distinct fingerprints of the isolates and multivariate statistical methods cluster type 1 parasites separately from mutually similar type 2 and type 3 parasites. We are currently analyzing which set of metabolites correlates with spontaneous stage conversions of these strains and will confirm these changes in targeted experiments on in vitro bradyzoites.

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126. Triazine nitriles targeting Cathepsin Protease L and chronic *Toxoplasma* infection

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Abstract. Zero effective treatment options exist for eliminating *Toxoplasma gondii* in infected people. Ocular patients are particularly at risk of progressive vision decline from recurring episodes of retinal damage. Absence of effective treatments also precludes testing potential contributions of chronic *T. gondii* infection to brain dysfunction. We reported recently that genetic ablation of the *T. gondii* lysosomal (VAC/PLV) cathepsin protease L (CPL) severely compromises *T. gondii* chronic infection both in culture and infected mice. Undigested material from parasite autophagy accumulates in the VAC/PLV of CPL deficient bradyzoites before they die, suggesting a critical role for processing of autophagic material during parasite persistence. Irreversible inhibition of CPL activity with a suicide inhibitor, LHVS, phenocopies genetic ablation of CPL. However, LHVS has poor drug-like properties and fails to enter the brain (and probably the retina) rendering it unsuitable for further development. Instead, we advanced a dipeptide nitrile series of compounds for potency and selectivity before scaffold hopping the optimized features to a triazine nitrile hit that we identified in a targeted high-throughput screen. Further optimization of the triazine series yielded CNS accessible compounds with low nanomolar potency for recombinant CPL and low micromolar efficacy for loss of bradyzoite viability in vitro. We continue to advance this series ahead of *in vivo* proof of concept treatment studies. Together this work highlights the feasibility of transitioning from genetic to pharmacologic studies for addressing chronic *Toxoplasma* infection as an important unmet medical need.

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127. A fern able to affect viability of *Toxoplasma gondii* in vitro.

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Abstract. *Pleopeltis crassinervata* (Fée T. Moore) is an Epiphyte fern from Mexico known as "lengua de ciervo" which is used in traditional medicine to disinfect mouth ulcers. Recent research indicates that extracts of this fern affects *Trichomonas vaginalis* axenic cultures (CI50 82.83 µg/mL. unpublished data). In the present work a bio-guided study was carried out. A methanol extract was obtained from the frond, which was fractionated. The Toxoplasmodicidal activity in vitro was determined by the viability of extracellular tachyzoites of *Toxoplasma gondii* of the RH strain, using the dye Sytox Green®. The active fraction was submitted to column chromatography and six compounds were obtained. The hexanic fraction of the methanol extract was the most active with a CI50 of 16.9 µg/mL, on the other hand, the compound #1 obtained from the hexanic was the most active compound with a CI50 of 23.69 µg/mL against the viability of tachyzoites at one hour of exposure. Research related to the effect of natural products against *T. gondii* Reports CI50 values ranging from 7 and even > 1000 µg/mL, so we consider the compounds obtained from the hexanic fraction as a Toxoplasmodicidal and are interesting to continue with future research in cell line, we are currently working on the chemical elucidation of the active compound. This work constitutes the first record of chemical-pharmacological investigations of *Pleopeltis crassinervata*.

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128. Resilience through disorder? Investigating LEA protein's potential to protect *Toxoplasma gondii* oocysts from stress

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Abstract. Four proteins specific to the oocyst stage of *Toxoplasma gondii* have been annotated as so called 'late embryogenesis abundant domain-containing proteins' (TgLEAs). Such proteins have characteristics of 'intrinsically disordered proteins'. Since proteins of this class only adopt a defined tertiary structure under certain physiological conditions, including stressors such as desiccation and low temperatures, our objective is to investigate whether TgLEAs also exhibit this characteristic and thus could contribute to oocyst survival under such environmental conditions. Bioinformatic and biochemical analyses were performed to substantiate TgLEAs' classification as intrinsically disordered proteins. Findings from gel filtration and western blotting of recombinantly expressed TgLEAs as well as algorithmic predictions strengthen our assumption that TgLEAs are highly intrinsically disordered proteins. Each of the TgLEA proteins was expressed in *Escherichia coli* in either wildtype strains or strains that carried mutations in certain stress related genes to investigate a possible beneficial contribution of individual TgLEAs on survival after stressing conditions such as desiccation, osmotic stress or extreme temperatures. Out of four investigated TgLEAs, at least one shows a beneficial effect on *E. coli* viability after desiccation, implying an involvement in desiccation tolerance of oocysts. TgLEAs that aid in protection against desiccation and extreme temperatures might contribute to the oocysts' resilience and the resulting long survival of *T. gondii* in the environment. Therefore, they could present potential targets for novel disinfectant strategies.

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SESSION V - GENOMICS, TRANSCRIPTOMICS AND EVOLUTION DISCUSSION LEADERS: DAVID ROOS- JESSICA KISSINGER**129. ToxoDB: The Functional Genomic Resource for *Toxoplasma gondii***

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Abstract. ToxoDB.org is an integrated functional genomics resource that provides free online access to genomic scale data. Data within ToxoDB is searchable via a graphical user interface that allows the development of complex in silico experiments to support hypothesis driven lab work. ToxoDB integrates a diverse array of data types from different sources including the underlying genomic sequences and annotations (32 different species and strains of *Toxoplasma*, *Cyclospora*, *Cystoisospora*, *Eimeria*, *Hammondia*, *Neospora*, *Sarcocystis*), transcript level data (RNA sequence, microarray and ESTs), protein expression data, epigenomic data (ChIP-chip and ChIP-seq), population-level (SNP) and isolate data, phenotype data (CRISPR). In addition, genomic analyses provide the ability to search for gene features, subcellular localization, motifs (InterPro and user defined), function (Enzyme commission annotation and GO terms) and evolutionary relationships based on gene orthology. Interactive metabolic pathways enable painting of experimental evidence on pathways and a user workspace enables analysis of primary data using Galaxy pipelines followed by private integration into ToxoDB. Highlights from ToxoDB: • Community annotation and curation via user comments. User comments including images, files, PubMed records, etc can be added to records in ToxoDB. • Graphical searches allow building complex multi-step strategies which can be saved, modified and shared. • Column analysis tools to generate word cloud graphics and histograms of results. • Search result analysis using GO or metabolic pathway enrichment. • User workspaces for primary data analysis and private integration into ToxoDB. *Presented on behalf of the entire EuPathDB team.

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130. High-resolution spatial proteome map of *Toxoplasma gondii*

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Abstract. Localising proteins in the cell is a major research strategy in apicomplexan cell biology, but the subcellular distribution of the majority of the proteome remains unknown, which drastically limits our understanding of apicomplexan biology. Here we present, for the first time in any apicomplexan, a high-resolution map of protein subcellular localisation in the *Toxoplasma gondii* extracellular tachyzoite. Using a spatial proteomics technology called hyperplexed Localisation of Organelle Proteins by Isotopic Tagging (hyperLO-PIT), we obtained proteomic evidence for over 5,000 proteins and generated a quantitative dataset for 3,832 proteins enabling high-throughput mapping of their subcellular location. Using a subset of 718 organelle marker proteins, we applied a supervised machine-learning classification method based on Bayesian mixture modelling and probabilistically attributed 1,913 of hypothetical and unknown proteins to 26 distinct subcellular niches. This includes novel microneme, rhoptry, dense granule, and apicoplast proteins. For select organelles (e.g., the mitochondrion, rhoptries, ER), we achieved sub-compartment resolution. For 62 unknown proteins, their hyperLO-PIT-predicted localisation was confirmed by gene tagging and fluorescence microscopy. This high-resolution spatial map provides highly robust organelle protein catalogues that greatly expand our understanding of the functional profile of subcellular compartments; reveals the distribution of novelty and conservation of organelle proteomes between apicomplexans and other eukaryotes; shows different rates of evolution across subcellular niches of the apicomplexan cell; and uncovers co-expressional patterns of proteins associated with the invasion-related compartments.

Funding. Isaac Newton Trust - Leverhulme Trust Early Career Fellowship to KB, KAUST Competitive Research Grant, MRC, Wellcome Trust.

131. High-throughput single cell sequencing to identify *Toxoplasma gondii* transcriptional effectors

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Abstract. *Toxoplasma gondii* secretes a large number of effector proteins into the host cell to facilitate infection, a key emerging class of which are transcriptional effectors that modulate host transcription to establish a favorable niche for parasite survival and replication. Examples of such transcriptional effectors include TgROP16, a rhoptry protein that inhibits inflammatory STAT signaling, and TgIST, a dense granule protein which suppresses interferon responses and thereby promotes parasite survival within the host cell. We recently developed an arrayed CRISPR library for targeted knock-out of large numbers of genes in a pool *Toxoplasma* tachyzoites. Using this library, we identified known and novel parasite virulence factors; however, we did not identify some of the proteins known to have a key role in reprogramming host transcription, suggesting that these knockouts may be complemented by other parasites in the pool. To identify effectors that drive transcriptional changes in the host cell, we are moving from a bulk *in vivo* setting to a single cell strategy, combining this selective knock-out screen with high throughput single cell RNA sequencing. Here we present the methodology and initial results from these experiments.

Funding. This work was supported by the Francis Crick Institute which receives its core funding from Cancer Research UK (FC001189), the UK Medical Research Council (FC001189), and the Wellcome Trust (FC001189).

132. Revaluation of the *N. caninum* and *T. gondii* genomes reveals large chromosomal rearrangements, misassembly and lack of synteny

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Abstract. With the exception of a few commonalities such as their obligatory intracellular lifestyle, apicomplexans differ greatly in host range specificity, pathogenicity and transmission strategy. Understanding the molecular basis of their speciation has been the focus of much attention. Some of these differences can be explained upon comparative genomes analyses. Api-genomes are small, ranging from 9 to 130 Mb, and shy of 900 genes are conserved among all. Major genomic rearrangements are observed. A stark exception are *N. caninum* and *T. gondii*, whose genomes have been proposed to be highly syntenic and largely conserved. Observable differences, between these parasites, in host specificity and host-parasite interactions have been explained largely by pseudo-genization and point mutations in a handful of virulence factors. However, our close examination of genomic data revealed that synteny had been artificially generated by a short-read sequence assembly. Advances in genome sequencing technologies have accompanied the fast paced genomics era. Third generation sequencing outperforms its parent technologies providing longer reads, hence, more readily assembled draft genomes. Re-sequencing and de novo assembly of two *Neospora caninum* genomes, using PacBio revealed previously unidentified genes, and underappreciated differences among *N. caninum* isolates. More importantly, major chromosomal rearrangements are observed between *T. gondii* and *N. caninum*, challenging the synteny paradigm. In addition, we re-sequenced and re-assembled the *T. gondii* genome, identifying large areas of inaccurate assembly. Importantly, we find that ChrVIII and ChrVIIa are in fact a single chromosome, reducing to 13 the number of chromosomes in both coccidia.

Funding. This project was funded by a grant from the National Agency for Research and Innovation (ANII) and the National Institute for Agricultural Research (INIA) number FSSA_X_2014_1_106026. MEF is funded by an ACIP grant from the International Pasteur Institute Network.

133. The Highly-Unusual yet Evolutionarily Conserved Mitochondrial Genome Sequence of the Coccidian *Toxoplasma gondii*

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Abstract. The mitochondrial genome sequence of many apicomplexan protist parasites is highly-reduced, ~6 Kb in length and consists of only three protein-encoding sequences and highly-fragmented large and small subunit ribosomal genes. Despite the apparent size and gene content conservation, the structures of the apicomplexan mitochondrial genome sequences identified to date have been variable, consisting mostly of linear and linear concatenation arrangements. Efforts to characterize the mitochondrial genome sequence of the coccidian parasite, *Toxoplasma gondii* have been hampered by the presence of thousands of random fragments of mitochondrial DNA (NUMT's) present in the nuclear genome preventing approaches utilizing sequence similarity like hybridization or PCR. Physical separation of the single *Toxoplasma* mitochondrion has also proven challenging due to its polymorphic nature. We have employed several physical and sequence-based approaches to determine 23 DNA sequence elements that constitute the *Toxoplasma* mitochondrial genome sequence. None of the elements encode a full gene. These sequence elements are highly-conserved in the related genera, *Hammondia* and *Neospora* and when combined in particular orders, are capable of encoding cob, coxI and coxIII as well as most of the SSU and LSU fragments found in other apicomplexans. The sequence elements exist in a variety of non-random permutations in which some elements are present more often than others. Multiple different molecules containing these sequence elements are observed. Cytochrome transcripts are detected. The physical structure of the genome sequence(s) remains elusive.

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134. Virulence Shift in a Sexual Clade of Type X *Toxoplasma* Infecting Southern Sea Otters

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Abstract. Fifty-three *Toxoplasma* isolates obtained from mustelids that stranded between 1998-2004 with toxoplasmosis (ranging from chronic infection to fatal encephalitis) were sequenced. ~74% of the sea otters collected throughout their geographic range were infected with Type X, a new genetic variant that has emerged in marine mammals. Depending on the locus investigated, Type X strains possessed one of only two allelic types that had independently assorted across the strains examined; either a genetically distinct allele or a Type II allele. Phylogenetic incongruence among locus-specific trees, genome-wide CGH array and WGS analyses confirmed that Type X is a sexual clade of natural recombinants that resemble F1 progeny from a genetic admixture; products of a cross between Type II and a mosaic of two distinct ancestries referred to as "gamma" and "delta". A single Type X genotype (19/53; 36%) had expanded in sea otters as chronic largely subclinical infections, but was highly pathogenic to mice (LD100 = 1 parasite). To determine whether murine virulence genes could be mapped within this naturally occurring population, we performed a genome scan and identified four QTLs with LOD scores greater than 3.8. Targeted disruption of ROP33, the strongest candidate from among 16 genes within the highest QTL on Chromosome VIIa established ROP33 as a murine virulence locus. The ability of this highly pathogenic Type X clone to expand and cause the majority of sea otter infections supports a virulence shift model whereby generalist pathogens like *Toxoplasma* utilize their sexual cycles to produce new strains with expanded biological potential.

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135. Tracing migration history of Japanese *Toxoplasma* population based on genome-wide SNP analysis

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Abstract. *Toxoplasma gondii* is a cosmopolitan parasite that infects virtually all warm-blooded animals including human. The sexual reproduction occurs in felid hosts; however, the parasites undergo mostly clonal proliferation and appear to be subdivided into 16 haplogroups (HGs). Previous studies showed that three HGs, which are supposed to adapt to domesticated animals, are predominant in European and North American continents. Recent accumulation of genotyping data revealed that a unique haplotype (HG13) predominates in China, again implying the association with the early expansion of civilization. Although the Japanese archipelago has been under the strong influence of the Chinese civilization, our genotyping study of Japanese isolates showed the presence of unique populations and the absence of HG13 parasites. To elucidate the fine-scale population structure and the relationship to neighboring populations, we performed high-throughput sequencing of eight Japanese isolates and estimated the divergence times based on the unique SNPs. One of eight isolates was almost identical to HG3 strains and supposed to be artificially introduced from the West after the Age of Exploration (15th-19th centuries). Three isolates constituted a local variant of HG2; however, they share the chimeric chromosome Ia with Chinese HG13. The molecular clock suggested that they diverged about 2,000 years ago, contemporaneously with the introduction of rice cultivation to Japan. The other four isolates were seemingly originated by the cross between the settled, agriculture-adapted population and indigenous, wildlife-adapted population. These results imply that the history of human being is also inscribed in the genomes of *T. gondii*.

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136. Understanding the role of mitochondrial-pellicle membrane contact sites in *Toxoplasma gondii*

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Abstract. *Toxoplasma's* singular mitochondrion is very dynamic and undergoes morphological changes throughout the parasite's life cycle. While intracellular, the mitochondrion is maintained in a lasso shape that stretches around the parasite periphery and is in close proximity to the pellicle, suggesting the presence of membrane contact sites. Upon egress, these contact sites disappear, and the mitochondrion retracts and collapses towards the apical end of the parasite. Once reinvested, the lasso shape is quickly reformed, indicating that dynamic membrane contact sites regulate the positioning of the mitochondrion. We discovered a novel protein (TgGT1_265180) that associates with the mitochondrion via interactions with the fission related protein Fis1. Knockout of TgGT1_265180, which we have dubbed Fip1 for Fission 1 Interacting Protein 1, results in a complete disruption of the normal mitochondrial morphology. In intracellular Fip1 knockout parasites the mitochondrial lasso shape is disrupted, and instead it is collapsed as normally only seen in extracellular parasites. Additionally, proper mitochondrial segregation is disrupted, resulting in parasites with no mitochondrion and extra mitochondrial material outside of the parasites. These gross morphological changes are associated with a significant reduction of parasite propagation and can be rescued by reintroduction of a wildtype copy of Fip1. We hypothesize that Fip1 mediates contact between the mitochondrion and the pellicle in a regulatable fashion, and that the Fip1-dependent morphodynamics are critical for parasite propagation. Current studies are focused on characterizing the consequences of mitochondrial collapse and identifying proteins that interact with Fip1 to position the mitochondrion to the periphery of the parasite.

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FRIDAY 21 JUNE 2019

SESSION VI - HOST PARASITE INTERACTIONS I**137. An alveolate conserved mechanism is implicated in rhoptry secretion in apicomplexa**

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Abstract. During apicomplexan parasites invasion, rhoptry proteins are injected in the host cells where they contribute to invasion and modulate host functions. Our knowledge on their exocytosis remains largely unknown. Ciliates and dinoflagellates, which are part of the Alveolata phylum together with Apicomplexa, contain also secretory organelles. The extrusive organelles of Paramecium, the trichocysts, have a characteristic ring of intramembranous particles with a central "rosette" of 8-10 larger particles on the membrane over the docking sites. Rosette assembly is required for exocytosis upon stimulation. Remarkably, a similar structure was observed at the apex of apicomplexan parasites, which has remained uncharacterized until now. The dissection of mutants lacking the rosette and defective for exocytosis (called ND for "non-discharge") prompted the identification of essential components of the exocytosis machinery in Paramecium. We identified and characterized the orthologs Nd6 and Nd9 in the model organism *Toxoplasma*. While TgNd6 localizes at the apical pole of the parasites, TgNd9 is located in the cytoplasm. Conditional depletion of TgNd6 and TgNd9 did not affect parasites intracellular replication, egress, or motility. While microneme secretion also remains unaffected, depletion of TgND6 or TgND9 abolishes rhoptry secretion and blocks invasion. Furthermore, freeze-fracture analysis showed a defect in rosette formation in the TgND9 mutant. Finally, taking advantage of the biology of another ciliate, Tetrahymena, we discovered a new protein, Nd10, conserved in

Alveolata and necessary for organelles exocytosis. This study identifies novel proteins essential for rhoptries secretion and, supports the hypothesis of an alveolate conserved mechanism for organelles secretion.

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138. Identification and functional analysis of IMC29, a novel daughter-enriched *Toxoplasma* IMC protein

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Abstract. The *Toxoplasma* inner membrane complex (IMC) is a peripheral membrane system that is composed of flattened alveolar sacs that underlie the plasma membrane, coupled to a supporting cytoskeletal network. The IMC plays important roles in parasite replication, motility, and host cell invasion. Despite these central roles in the biology of the parasite, many of the proteins that constitute the IMC remain unknown, particularly those are important for the early stages of daughter cell formation. We have adapted the technique proximity-dependent biotin identification (BioID) for use in *T. gondii* to identify novel components of the IMC, uncovering over 45 new IMC proteins in both the alveolar and cytoskeletal sub-compartments. Importantly, labeling of IMC proteins using this approach has revealed a group of proteins that are enriched in the daughter cells. One of these daughter-enriched proteins (IMC29) is predicted to have a severe phenotype by the *Toxoplasma* genome wide CRISPR screen. In agreement with this, disruption of IMC29 by CRISPR/Cas9-induced homologous recombination results in significant replication defects as well as gross abnormalities in extracellular parasites. These defects in vitro translate to a substantial loss of virulence *in vivo*. Functional complementations with IMC29 deletions are in progress to identify IMC targeting elements and to determine which regions are important for function. These results suggest that IMC29 plays a critical scaffolding role in the formation of daughter cells during endodyogeny. Collectively, our study promises to provide new insights into the function of daughter-specific IMC proteins and provide new parasite-specific targets for therapeutic intervention.

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139. ROP55, a new rhoptry protein that subverts host cell response and mediates virulence of *Toxoplasma gondii*

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Abstract. The ability of *Toxoplasma* to cause disease depends on the coordinated secretion of specialized secretory organelles, namely micronemes, rhoptries and dense granules. During invasion, rhoptry proteins are injected in the host cell contributing to the invasion process but also hijacking host functions crucial to establish and maintain infection. Here we describe a new rhoptry protein, ROP55, which is located at the parasitophorous vacuole membrane. A knock-out line was generated, showing the non-essential role of this protein in vitro. The growth of KO-ROP55 parasites in human fibroblasts is significantly reduced and the mutant is highly attenuated *in vivo*, killing mice only after injection of one-million parasites. The deletion of ROP55 does not affect parasite invasion, egress, or replication; however the number of vacuoles after one day of infection is reduced, as well as the number of lysis plaques after 7 days of infection. This phenotype is fully restored in a strain ectopically expressing ROP55 and suggests that KO-ROP55 parasites die within the host cell. We then quantified survival of parasites either during serum starvation or in presence of IFN γ , and concluded that the phenotype observed was not due to a defect in nutrient import or IFN γ susceptibility. Since *Toxoplasma* is known to induce the inflammasome response, we conducted preliminary experiments and showed an increase in both IL-1 β and NLRP3 transcripts in cells infected by KO-ROP55. *In vivo* and in vitro studies are ongoing to further determine the involvement of ROP55 in the regulation of the cellular innate response against *Toxoplasma* infection.

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140. Preventing cellular senescence as a means to promote parasite replication and suppress inflammatory responses: new insights from the *Toxoplasma/Hammondia hammondi* system.

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Abstract. *Toxoplasma gondii* and *Hammondia hammondi* are closely-related coccidian intracellular parasites with distinct life cycle characteristics and ability to cause disease. *T. gondii* is capable of infecting all warm-blooded animals and humans where it can be highly pathogenic. In contrast, *H. hammondi* has a narrower host range and is not known to cause severe disease in animals or humans. To better understand virulence differences between these species we first performed a transcriptomic analysis of the response of a monocyte cell line (THP-1) to these two parasite species. We found that *T. gondii* and *H. hammondi*-infected cells had unique transcriptome profiles, with *H. hammondi* inducing significantly higher levels of proinflammatory cytokine production and *T. gondii* being capable of suppressing this effect. Using pathway analyses we found that *H. hammondi*-infected cells were in a cellular state that was consistent with cellular senescence while *T. gondii*-infected cells were not. We have confirmed this using flow cytometric cell cycle analyses which indicate that while *T. gondii*-infected cells progress through the S-phase into G2/M, *H. hammondi*-infected cells arrest in G1 and display a Senescence Associated Secretory Phenotype (SASP). We are now investigating how *T. gondii* suppresses SASP during infection and the impact of the cellular senescence on the replication rate of *T. gondii* and *H. hammondi*.

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141. Neurons clear *Toxoplasma gondii* through interferon- γ mediated mechanisms

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Abstract. In the CNS *T. gondii* cysts are primarily found in neurons. Previous in vitro work suggested that this enrichment in neurons was because IFN- γ -stimulated neurons were unable to restrict *T. gondii*. Using a system in which parasites trigger host cell expression of a green fluorescent protein (GFP) via injection of a rhoptry protein (Toxofilin) fused to Cre-recombinase (TCre), we have shown that during *in vivo* infections, *T. gondii* almost exclusively interacts with neurons and that most of these neurons are not actively infected. This finding of uninfected neurons suggests that neurons may be able to clear parasites. However, as TCre is injected prior to invasion, these uninfected GFP+ neurons could also arise from injection without invasion. To address the question of whether neurons are capable of clearing parasites *in vivo*, we developed a new reporter system by fusing Cre to Gra16 (GCre), a secreted effector protein that is released into host cells after invasion. To date, our studies using primary neuronal cell cultures and GCre parasites have shown that: i) neurons respond to IFN- γ ; ii) GCre is capable of Cre-mediated recombination in neurons; iii) 97+% of neurons that have undergone Cre-mediated recombination via GCre are actively infected; and iv) IFN- γ -stimulation of neuronal cultures prior to infection reduces this percentage to 66-84%, consistent with clearance of intracellular parasites. Current work is focused on determining if neurons can clear cysts in vitro and infecting Cre-reporter mice with GCre parasites to determine if neurons clear intracellular parasites *in vivo*.

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142. Chronic *Toxoplasma gondii* elicit transcriptional changes in host cells to prevent IFN gamma-mediated cell death

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Abstract. *Toxoplasma* tachyzoites extensively manipulate their host cell by exporting a distinct repertoire of effector proteins across the newly-established parasite vacuole (PV). This process interferes with the host transcriptional program, and is thought to enable parasite persistence and dissemination in spite of the host immune response. Eventually, *Toxoplasma* establishes a chronic brain infection that becomes a reservoir for disease reactivation and is seemingly drug resistant. Little is known about how this chronic stage of infection persists post-cyst formation, or whether protein export and host manipulation play a role in latency. Our research shows that in vitro bradyzoite-cysts drastically alter the host transcriptional program via effector protein export mediated by the dense granule PV membrane protein, MYR1. We identified TgIST, an inhibitor of host IFN gamma signalling, as the only known tachyzoite effector to be exported during bradyzoite stages, suggesting a role for TgIST in chronic infection. Furthermore, we demonstrate that protein export is critical for protecting bradyzoite infected host cells from undergoing IFN gamma-mediated cell death, thus enabling cyst persistence. This work provides the first evidence of the mechanism used by *Toxoplasma* bradyzoites for their long-term survival and identifies MYR1 as a potential drug target for the clearance of chronic toxoplasmosis.

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SESSION VII - IMMUNOLOGY II

143. Persistent *Toxoplasma* infection of the Brain Induced Neurodegeneration associated with Activation of Complement and Microglia

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Abstract. *Toxoplasma gondii*, a common neurotropic parasite, is increasing being linked to neuropsychiatric disorders including schizophrenia, Alzheimer's disease, and Parkinson's disease. However, the pathogenic mechanisms underlying these associations are not clear. *Toxoplasma* can reside in the brain for extensive periods in the form of tissue cysts, and this process requires a continuous immune response to prevent the parasite reactivation. Because neuroinflammation may promote the onset and progression of neurodegenerative diseases, we investigated neurodegenerative-associated pathological changes in a mouse model of chronic *Toxoplasma* infection. Under conditions of high-grade chronic infection, we documented the presence of neurodegeneration in specific regions of the prefrontal cortex, namely, the anterior cingulate cortex (ACC) and somatomotor cortex (SC). Neurodegeneration occurred in both glutamatergic and GABAergic neurons. Neurons that showed signs of degeneration expressed high levels of CX3CL1, were marked by profoundly upregulated complement proteins (e.g. C1q and C3), and were surrounded by activated microglia. These results suggest that chronic *Toxoplasma* infection leads to cortical neurodegeneration and that resulting CX3CL1, complement and microglial interactions which mediate the phagocytic clearance of these degenerating neurons. Our study provides a mechanistic explanation for the link between *Toxoplasma* infection and psychiatric disorders.

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144. The naïve CD8 T cell IFN γ response is intersected by multiple to *T. gondii* effectors and the host's inflammasome

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Abstract. Host survival to *Toxoplasma gondii* infection is dependent upon CD8 T cell IFN γ responses. Since manipulation of CD8 T cells may influence *T. gondii*'s ability to achieve chronic infection, we asked whether the parasite modulates activation of this cell type. To address this, we analyzed naïve CD8 T cell responses to the endogenous, vacuolar resident antigen, "TGD057". Naïve TGD057 antigen-specific CD8 T cells were isolated from transnuclear mice and assayed for their ability to secrete IFN γ to *T. gondii*-infected bone marrow

derived macrophages. A unique phenotypic pattern emerged in which CD8 T cells responded vigorously to all *T. gondii* strains, except those from clade A, suggesting the presence of a polymorphic Regulator Of CD8 T cell Responses (ROCTR). Genetic mapping implicates at least two ROCTR candidates are encoded on *T. gondii* chromosomes X and XII, and a third ROCTR, which controls the early differentiation of IFN γ + CD8 T cells at 14 hours, is on chromosome VIIIb. The CD8 T cell IFN γ response to TGD057 was independent of the parasite's protein export machinery Myr1, but modulated by regulators of dense granule parasitophorous vacuole membrane (PVM) association, including ASP5, GRA42 and GRA43. Because this phenotype requires host expression of the inflammasome and IRG (Immunity-Related GTPases) pathways, we propose a two-step mechanism in which a first set of ROCTRs modulates PVM integrity for antigen escape, and a second set of ROCTR(s) intersect the host's inflammasome pathway to influence CD8 T cell IFN γ differentiation through the IL-1/-18 axis.

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145. Human chronic toxoplasmosis is accompanied by changes in the phenotype and reactivity of PBMC-derived monocytes

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Abstract. Primary infection with *Toxoplasma gondii* leads to robust cell-mediated immune responses, which control but do not clear the infection, thus enabling long-term parasite persistence in brain and muscle tissues. Chronic toxoplasmosis in mice is associated with resistance to heterologous pathogens and this has been related to increased numbers of inflammatory monocytes. Here, we determined phenotypes of monocytes from healthy human blood donors chronically infected with *T. gondii* or *T. gondii*-negative, and we unravelled monocyte responses in vitro. CD14+ monocytes from *T. gondii*-seropositive individuals expressed significantly less CD16 as compared to those from seronegative controls. Furthermore, the percentages of CD62L+ and CD64+ monocytes were lower and higher, respectively, among PBMCs from individuals with chronic toxoplasmosis compared to those from controls. Chronic toxoplasmosis was however not associated with a significant shift in the distribution of classical, intermediate and non-classical monocyte subpopulations. In vitro infection of monocyte-enriched PBMCs from both seropositive and seronegative blood donors with *T. gondii* led to an expansion of CD14+ classical monocytes and a decrease of CD14+CD16+ monocytes. Furthermore, the percentages of CCR2+ monocytes strongly decreased after infection. Only monocytes from chronically infected individuals but not those from naive controls dose-dependently up-regulated MHC class II expression following in vitro infection. Finally, 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 seronegative controls. Thus, infection of humans with *T. gondii* leads to long-term effects on the phenotype and the reactivity of monocytes.

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146. Vascular remodeling and trafficking of intracellular parasites revealed by intravital imaging of the brain during *T. gondii* infection

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Abstract. *T. gondii* has the remarkable ability to breach the blood-brain barrier (BBB) and establish infection in the CNS. Evidence indicates that *T. gondii* can infect and lyse endothelial cells at the BBB for entry to the CNS in mice.

However, little is known about the effects of infection on the BBB. To investigate *T. gondii* interactions at the BBB and barrier regulation *in vivo*, we installed cranial windows in transgenic mice expressing the tight junction protein claudin-5 fused to eGFP in endothelial cells. We then performed longitudinal two-photon intravital imaging in these mice during the course of infection. We observed profound vascular remodeling during acute infection, which was characterized by increased tortuosity of individual endothelial cells and reorganization of the vascular network. Endothelial cell cytoskeletal remodeling was also observed in *in vitro* experiments of primary human endothelial cells infected with *T. gondii* and cultured in conditions of physiologic shear stress. In addition, two-photon imaging through the cranial windows of infected mice revealed both individual tachyzoites and vacuoles of *T. gondii* within highly motile cells travelling along cerebral blood vessels. These vacuoles were highly dynamic and traveled at speeds greater than 5 μ m/min. Flow cytometry of brain homogenates from infected mice demonstrated infiltrating peripheral immune cells harboring parasites, as well as infected microglia. Collectively, these data reveal marked changes in the cerebral vasculature during *T. gondii* infection and the detection of motile, infected cells trafficking the parasites through the CNS.

Funding. We thank members of the Lodoen, Tenner, Nelson, Morrissette, Andrade, and Messaoudi labs for helpful discussion on this project. We thank Dr. Adeela Syed of the Optical Biology Imaging Core Facility at UCI for her expertise in imaging, Dr. Dritan Agalliu for providing the claudin-5 eGFP mice. This work was supported by NIH NIAID R01AI120846 (to M.B.L.), American Cancer Society RSG-14-202-01-MPC (to M.B.L.), and NIH678 NIAID T32 for Training in Immunology (to C.A.S. and E.M.H.).

147. Immunity to *Toxoplasma gondii* -Nfkbid and the antibody response to the GPI anchor

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Abstract. A fully protective vaccine is currently unavailable for any human parasitic pathogen. Among parasites that evade vaccine-induced immunity is *Toxoplasma gondii*. We previously reported that South American strains are particularly adept at immune evasion and cause lethal secondary infections in vaccinated or chronically infected mice. To understand whether immunity could be generated against virulent strains, we turned to mouse genetics. Unlike susceptible C57BL/6J mice, we noted A/J mice were highly resistant to secondary infection and therefore, determined resistance loci that segregated within 26 recombinant inbred (AxB, BxA) lines. The QTL with largest effect encodes a highly polymorphic gene, Nfkbid, and found that Nfkbid null mice (bumble) were susceptible to secondary, but not primary infection. Bumble mice had intact memory T cell responses, but failed to generate parasite-specific IgM and were defective in producing most parasite-specific IgG isotypes following infection. Nfkbid encodes I κ BNS, which belongs to a family of nuclear NF- κ B regulators, and is required for B-1 cell development and T-independent antibody responses. Consistent with this, nearly 70% of the antibody reactivity in primed mice is directed against the glycoposphatidylinositol (GPI)-anchor of *T. gondii*, a molecular structure that covers the surface of most protozoan parasites and is a known B-1 cell antigen. Taken together, we propose a model in which T cell-mediated immunity to *T. gondii* must be 'layered' with Nfkbid-driven B-1 cell responses to the GPI-anchor of *T. gondii*. Antibody responses to non-protein antigens may be fundamental for immunity to most parasites, and in theory, should be targeted in parasite vaccines.

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148. Cachexia is a cost of long-term reliance on innate immune tolerance programs in chronic *Toxoplasma* infection

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Abstract. *T. gondii* is a protozoan parasite that causes lifelong infections in many warm-blooded organisms, including humans and mice. An intact immune response is necessary to restrict parasite replication throughout infection. Here we show that mice infected with *T. gondii* can develop a robust and sustained form of cachexia. Cachexia is an immune-metabolic disease of progressive muscle wasting that impairs survival and quality of life across a wide

range of chronic diseases. Consistent with an emerging role for IL-1 signaling in tolerance to inflammatory damage, mice deficient in the Type 1 IL-1 receptor (IL-1R^{-/-}) control parasite growth, but have more severe acute disease pathology than wildtype mice. However, IL-1R^{-/-} mice rapidly recover from acute muscle wasting and have significantly increased survival compared to wildtype mice. Tissue remodeling is an important adaptation to the vasodilation, edema and cell death induced by an immune response. In *T. gondii* induced cachexia, wildtype mice do not recover from acute myofibroblast activation and develop multi-organ perivascular fibrosis. The progression to fibrosis is dependent on IL-1R and IL-1 is sufficient to directly induce myofibroblast differentiation in vitro. These data are consistent with a model where IL-1R signaling is beneficial to tissue integrity over short periods of inflammation, but in the setting of chronic inflammation, sustained reliance on IL-1 mediated tolerance programs comes at the cost of fibrosis and cachexia.

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SESSION VIII - CLINICAL TOXOPLASMOSIS

149. Point-of-care test for anti- *Toxoplasma* antibodies: a Novel paradigm

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Abstract. Point-of-care (POC) tests detecting *Toxoplasma gondii* infection offer solutions, potentially addressing cost concerns and leading to better clinical outcomes through improved access to screening. Testing performance of a lateral flow immunochromatography-based *Toxoplasma* ICT IgG-IgM test for combined detection of *Toxoplasma*-specific IgG and IgM has been previously described using serum samples from the National Collaborative Chicago-Based Congenital Toxoplasmosis Study (NCCCTS) and other cohorts. Herein, we tested whether a comparably performing whole-blood-variant test (designated "BK") could perform with high sensitivity and specificity, obviating the need for venipuncture and sample processing infrastructure and making an efficient, low-cost POC test. Samples were obtained from consenting United States individuals, including seropositive individuals affiliated with the NCCCTS and obstetrical patients in Chicago, and obstetrical patients in Morocco. A total of 244 samples from 205 consenting individuals, including seropositive individuals, obstetrical patients in the U.S., and obstetrical patients in Morocco was examined. Of these, 143 samples were seronegative for *T. gondii* and 101 were seropositive (defined as having detectable anti-*Toxoplasma* IgG and/ or IgM). Our work demonstrates that the *Toxoplasma* ICT IgG-IgM test can function reliably as a point-of-care test to diagnose *Toxoplasma gondii* infection in the U.S. This provides an opportunity to improve maternal-fetal care by using approaches, diagnostic tools, and medicines already available from the present study, it appears a simple, low-cost POC test is now available to help prevent morbidity/disability, decrease cost, and make gestational screening feasible. It also offers new options for improved prenatal care in low- and middle-income countries.

Funding. NIH Traher Foundation

150. An alternative method to obtain specific IgM *T. gondii* peptides to be used as diagnostic tools.

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Abstract. Toxoplasmosis is an infectious disease caused by the parasite intracellular protozoa *Toxoplasma gondii* (*T. gondii*). Although it is usually asymptomatic in healthy individuals, it can be severe in immunosuppressed patients and in congenital infections being able to cause abortion or neonatal malformations. This situation emphasizes the need of distinguish between primary and chronic infections. Acute toxoplasmosis diagnosis is based mainly on serological tests that detect IgM anti-*T. gondii* antibodies. For decades, commercial kits were based on *Toxoplasma* lysate antigens (TLA) obtained from infected mice and / or tissue cultures. Sometimes the use of these TLA presents inherent limitations in terms of performance and strong regulation. Nowadays, purified recombinant antigens have become an interesting alternative with proved diagnostic utility. However, these techniques, despite ha-

ving great advantages, are time-consuming and cost-expensive. The objective of the present work was to obtain specific IgM *T. gondii* epitopes using solid phase peptide synthesis (SPPS) as an alternative to purified recombinant antigens. In addition, these epitopes were evaluated as capture antigens to detect specific antibodies against *T. gondii*. Peptide-microarray analysis was used to identify the specific sequences for the detection of IgM anti-*T. gondii*. Initially, twenty peptides from eight immunoreactive proteins and subsequently seven synthetic structures based on different epitope combinations were selected and synthesized by SPPS, then purified by Reverse-Phase HPLC and analyzed by Liquid Chromatography-Mass Spectrometry. To assess the ability of these peptides to detect anti-*T. gondii* IgM we performed both multiplex-ELISA additionally with chemiluminescent-based immunoassay with surface-functionalized paramagnetic microparticles using the fully automated benchtop analyzer BIO-FLASH®.

Funding. This project is being developed thanks to the invaluable contribution of many people from Biokit and from the Proteomics and Protein Chemistry Unit. The authors would also like to acknowledge infNity-biomarkers for their contribution with the peptide evaluation. This work is part of an industrial thesis project, partially financed by Industrial Doctorate Program and the Agency for Management of University and Research Grants of the government of Catalonia (AGAUR).

151. Standardization of an ELONA assay for *Toxoplasma gondii* ROP18 detection in Human Serum Samples

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Abstract. ROP18 is a major virulence factor of *Toxoplasma gondii*. There are not any methods that allow the direct detection of the protein in human serum; also, it is unknown if ROP18 could be related to the clinical manifestation. Therefore, in this work we evaluated in silico the affinity of 2 DNA aptamers (AP2039 and AP2056) for ROP18 and standardized a direct ELONA assay to detect ROP18 in human serum from individuals with toxoplasmosis. The positive controls for the ELONA included total antigen of *T. gondii* RH and a recombinant version of ROP18 (rROP18). The ELONA assay was used to evaluate 62 seropositive samples with different clinical forms of toxoplasmosis and 20 seronegative samples. Molecular docking of both aptamers with ROP18 was performed using AutoDock-Vina 1.1.2 and the software Chimera to visualize the docked structures. The ELONA assay showed that AP2039 presented higher values over AP2056 in terms of affinity and specificity for rROP18 (AP2039-Kd = 62,7 ± 17,2 nM vs AP2056-Kd = 97,7 ± 22,2 nM). AP2039-ELONA was positive for 22.6% (14/62) of the samples with toxoplasmosis. A significant association between the test positivity and congenital toxoplasmosis (p=0,006) was found. Docking analysis for the affinity of AP2039 for ROP18 showed a better binding energy (-10.2 Kcal/mol) compared with AP2056 (-9.4 Kcal/mol). Finally, conditions of an ELONA assay for *Toxoplasma*-ROP18 were standardized. This ELONA assay showed that test positivity is correlated with congenital toxoplasmosis. Experimental and in silico analysis showed that aptamer AP2039 could be used as a recognition agent to detect ROP18 in human serum samples with toxoplasmosis.

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152. Apolipoprotein A1 May Play A Role in Protection Against *Toxoplasma gondii*: Potential for A New Biomarker?

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Abstract. There is clinical utility in the identification of biomarkers which might identify those patients at most significant risk for the development of severe toxoplasmosis. Such individuals might be monitored more closely or merit suppressive therapy given the chronic nature of the infection. *T. gondii* is an obligate scavenger of host cholesterol for basic metabolic processes, and thus, the handling of cholesterol by the host could be of significance to the interaction between the host and this parasite. Apolipoprotein A1 (ApoA1) is an important component of high-density lipoprotein (HDL), a carrier of cho-

lesterol in humans. This lipoprotein has been implicated in numerous pathophysiological and physiological processes. Additionally, there is a biological precedent for the involvement of lipoproteins in protection against pathogens. For example ApoL1 has been demonstrated to have cytolytic activity against *Trypanosoma brucei*, the causative agent of African Sleeping Sickness. Given the known integral importance of apolipoproteins to host metabolism and their appreciable interface with pathogenic organisms, it was, perhaps, unsurprising to identify a 5-fold increase of ApoA1 in the serum of vaccinated mice. Herein, we present the initial characterization of the role of host ApoA1 in the pathogenesis of toxoplasmosis, with murine models of infection as well as clinical correlation from samples from congenitally infected humans.

Funding. NIH

153. Evaluation of therapeutic failure, adherence to treatment and adverse effects in ocular toxoplasmosis

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Abstract. Purpose: To determine the frequency of therapeutic failure, adverse effects and poor adherence to treatment in patients with ocular toxoplasmosis. Materials and methods: Retrospective descriptive study. Patients of all ages diagnosed with ocular toxoplasmosis, with follow-up of at least 35 days after the start of systemic treatment for the active episode, assessed in three Ophthalmological reference centers in Colombia. Results and discussion: 140 patients were characterized with an average age of 31,17 with SD of $\pm 15,99$ years, 78 women and 62 men. The main reasons for consultation were blurred vision (88,03%) and myodesopsia (46,15%). The outcomes measured were: improvement in VA (55,45%), improvement of cellularity in the anterior chamber (96,67%) and vitreous inflammation (68,63%), and the decrease of the size of the retinocoroid lesion (80,46%). Therapeutic failure was presented in 31,43% during the initial treatment. The initial schemes used were compared in these patients, finding that those treated with Pyrimethamine-Sulfadoxine presented a lower frequency of therapeutic failure (17,50% $p = 0.05$) compared to Trimethoprim-Sulfamethoxazole (37,29% $p = 0.06$). The most frequent adverse effects were diarrhea, maculo-papular rash and pruritus (2.56% each). Severe adverse reactions such as Steven Johnson and pseudomembranous colitis occurred only in one patient each. Poor adherence to treatment was observed in 6 (4.29%) of patients, however, it was not related to the adverse effects of the medication and three of them presented therapeutic failure. Conclusion: Patients with ocular toxoplasmosis treated with Pyrimethamine-Sulfadoxine as first-line therapy, presented lower percentage of therapeutic failure than those treated with Trimethoprim-Sulfamethoxazole.

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154. Evaluation of the noninvasive imaging method spectral domain optical coherence tomography (sd-oct) in toxoplasmic retinochoroiditis patients

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Abstract. Introduction: SD-OCT is a non invasive test that evaluate morphological changes in the retina, vitreous, and choroid during retinochoroiditis and other ocular diseases. Methods: We evaluate the imaging of 31 patients attended and treated in the Retinopathy Clinic with eye injury as result of a *T. gondii* infection. Serology determine the infection and SD-OCT the healing process of the eye injury. Results: All the patients were IgG positive for toxoplasmosis. The average age of patients was 35 years old, with a predominance of male patients than females. The retinochoroiditis were identified by funduscopy and by the SD-OCT exams characterizing the eye injuries, identifying the retinal folds and the macular region features, as well as the retinal pigment epithelium features, such as atrophy and hypertrophy. Discussion and Conclusion: The growing interest in toxoplasmosis has helped to develop strategies for early clinical intervention. Imaging tests such as SD-OCT helps in

the assessment of the eye disease showing whether it is in the acute phase or quiescent, and can also be used to monitor eye involvement in respect to *T. gondii* infection and is a non invasive effective tools to tracking of the healing process of the retinochoroiditis caused by *T. gondii*.

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155. In vitro studies on type 1 and 3 interferons in ocular toxoplasmosis

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Abstract. Ocular toxoplasmosis (OT) is responsible for most of posterior uveitis worldwide, with a peculiar pathophysiology involving the persistence of parasites in infected tissues and sporadic recurrences of the infection. Interferon (IFN) γ is known to be a critical factor of the innate immune response during OT, but little is known regarding other kinds of IFNs in this infection, especially of types 1 (α and β) and 3 (λ 1, 2 and 3). The aim of our study was to assess the role of these IFNs in vitro. By infecting cultures of retinal cells, we evaluated the inhibition of parasite multiplication following stimulation with recombinant type 1 and 3 IFNs. We also assessed the expression of type 1 and 3 IFNs and their receptors, as well as IFN γ and the well-known IFN γ induced effector mechanisms in human immune response to *T. gondii* infection, indoleamine 2,3-dioxygenase (IDO) after infection. We used two different strains of *T. gondii*, one virulent (RH), the other avirulent (PRU). We observed significant inhibition of parasite proliferation upon stimulation of microglia with IFN β and IFN λ . Remarkably, the inhibitory effect of IFN λ was of similar magnitude as the effect of IFN γ . We observed that the infection of retinal cells resulted in the expression of IFN β and IFN λ 1, 2 and 3. Our results suggest that type 1 and type 3 IFNs may be involved in the innate immune response to OT, in a cell type specific way. These first results will be further tested in our OT mouse models.

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SESSION IX - TRAFFICKING PATHWAYS

156. Characterization of protein effector export in the bradyzoite stage of *Toxoplasma*

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Abstract. The bradyzoite life-stage of *Toxoplasma* is responsible for maintaining a chronic infection in its host, during which semi-dormant parasites persist predominately in the central nervous system. Little is known regarding if and how bradyzoites, residing in modified vacuoles known as tissue cysts, manipulate their host cell so as to maintain a persistent infection. Our proteomic studies of the bradyzoite cyst wall, an amorphous structure that is formed at the membrane of the bradyzoite parasitophorous vacuole, identified MYR1 as a component of this structure. Given the function previously reported for MYR1 in tachyzoite vacuoles, our finding suggested that the export of proteins involved in host-parasite interactions may also occur in tissue cysts. We sought to determine whether bradyzoites, either during the course of differentiation or upon infection of a new host cell, retain the capacity to translocate proteins via this pathway. Four known protein effectors that translocate from the parasitophorous vacuole into the host cell nucleus (GRA16, GRA24, GRA28, TgIST) were epitope tagged at their endogenous loci in the Type II Prugnialud strain. Immunofluorescence of infected fibroblast cultures under bradyzoite growth conditions demonstrated that these effectors translocate across the developing cyst wall at early, but not late, timepoints post-infection. This pattern was seen during both early tachyzoite to bradyzoite transition or after bradyzoite invasion of new host cells. This work has been further extended to characterizing effector export in vitro in primary neuron cultures, as well as visualizing parasite effector export *in vivo* in murine brain during acute and chronic infection.

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157. It Takes a Village: At Least EIGHT Proteins Are Required for Translocation of GRA Effectors Across the Parasitophorous Vacuole Membrane.

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Abstract. Many studies have shown that *Toxoplasma* is in active dialogue with the host cell and that the parasites introduce a large number of effector proteins into the host cell. These effectors come in two flavors – ones that originate in the apical secretory organelles known as rhoptries and that are injected during invasion (ROPs); and a second set that are secreted into the parasitophorous vacuole (PV) from dense granules after invasion (GRAs). I will describe our recent efforts to understand the translocation of GRA effectors across the PV membrane (PVM). We now know of a total of 8 proteins that are all required for this translocation phenomenon: one is a Golgi-resident protein, ASP5, while another is a rhoptry protein kinase, ROP17, that acts catalytically on the host-cytosolic side of the PVM. The remainder are themselves dense granule proteins that appear to form an extensive complex within the PVM. I will present the identification of this complicated machinery, the extent to which the different members interact and our current understanding of the roles that at least some of them play in this crucial part of intracellular life.

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158. Identifying host metabolic pathways that regulate *Toxoplasma* growth

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Abstract. Following the establishment of a replicative niche in the cytosol, *Toxoplasma* requires nutrients to grow—the same nutrients that host organelles need for their biogenesis and to perform metabolic processes. *Toxoplasma* must therefore compete with organelles for these nutrients. The organelle-*Toxoplasma* competition for metabolites is a fundamental, but little understood aspect of the host-pathogen interaction. Previous work from our lab and others have shown that *Toxoplasma* acquires fatty acids derived from lipid droplets, and that mitochondria fuse to enhance their fatty acid uptake, limiting *Toxoplasma* access to a key host resource and thus restricting its proliferation. Here, we continue to probe the role of host metabolism using a genetic screen to identify host factors that regulate *Toxoplasma* growth.

Funding. Max-Planck-Gesellschaft

159. K13 homolog in *Toxoplasma* associates with endocytic adaptors and a pore in the inner membrane complex

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Abstract. Artemisinin and its derivatives are currently the most widely used antimalarial drugs, however, resistant *Plasmodium* strains are recently spreading. In South East Asia most of the mutations in the resistant strains have been mapped to a single gene, which encodes a kelch-domain protein called K13. In Africa, on the other hand, drug resistant phenotypes have been associated with mutations in a component of the AP2 adaptor complex, AP2 μ . Despite the high interest that K13 has provoked in the field of malaria research, its function is still poorly understood. A homolog of K13 is present in *Toxoplasma gondii*, and we have sought to understand the behaviour and function of TgK13 with the potential to shed light on its function in both parasite systems. We determine that TgK13 specifically localizes to distinct spots in the cell pellicle and that it associates with components of the endocytic machinery including subunits of the AP2 adaptor complex, Eps15-homology domain containing protein and a dynamin-like protein. Using super-resolution microscopy, we found that K13 forms a ring-like structure that is stably associated with a pore spanning the cell pellicle

of *Toxoplasma* including both the IMC and alveolin subpellicular network. A dedicated and stable site for endocytosis likely represents an evolutionary solution to the problem of transporting nutrition from the host cell into the parasite, which is otherwise shielded by the pellicle. The implication of this structure in formation of artemisinin-resistant *Plasmodium* suggests that modulation of endocytosis is the mechanistic basis of tolerance to this drug.

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160. An unconventional myosin controls the positioning of the endosome-like compartments in *Toxoplasma gondii*

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Abstract. Sequential release of proteins from the secretory organelles (micronemes and rhoptries) is critical for *Toxoplasma gondii* host cell invasion. Microneme and rhoptry proteins are synthesized in the ER and must be sequentially trafficked through a highly polarized endomembrane system, in order to reach their final destination in the mature secretory organelles. The strict spatial positioning of endosomal compartments is thought to optimize the trafficking of proteins this pathway. However, little is known about how the parasite cytoskeleton controls the positioning of these organelles or the transport of vesicles between these compartments. Our data demonstrate that an unconventional myosin, TgMyoF, and actin control the apical positioning of the endosome-like compartments (ELCs). In control parasites NeonFP-Rab5, EmeraldFP-Rab6, Neon-Rab7, DrpB-GFP and GFP-syntaxin-6 all localize, as expected, to distinct post-Golgi compartments. Live cell imaging demonstrates that the Rab5 and Rab6 compartments are highly dynamic and vesicles can be visualized budding from and fusing with these compartments. In addition, Rab5- and Rab6- positive vesicles exhibit directed motor-driven motion with velocities of $1.54 \pm 0.03 \text{ mm/s}$ and $1.01 \pm 0.02 \text{ mm/s}$ respectively. Upon TgMyoF knockdown (KD) or actin depolymerization with cytochalasin D, the ELCs became fragmented, static and distributed through the cytosol. TgMyoF-KD resulted in a 50% decrease in the number of directed transport events, while actin depolymerization resulted in an almost complete inhibition (>95%) of directed transport. TgMyoF KD results in a 50% decrease in parasite invasion and we hypothesize that this invasion defect is due to the sub-optimal trafficking of proteins through the endosomal pathway in the absence of TgMyoF.

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161. Lipid asymmetry and SNARE associated proteins drive secretory organelles fusion and membranes biogenesis in *Toxoplasma gondii*.

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Abstract. Cellular membranes display specific and diverse functions conferred by a defined composition and organization of proteins and lipids. The asymmetric distribution of phospholipids (PLs) across the plasma membrane, trans-Golgi network and endosomal system of eukaryotic cells creates physical surface tension that is used to induce membrane curvature, favoring vesicle budding and fusion, or transduction of biochemical signals. These PLs gradients are set and maintained by different groups of proteins including flippases (FLIPs), which form hetero-complexes with CDC50 proteins acting as cofactors. These P4-ATPase proteins couple the hydrolysis of ATP with inward PLs translocation. The genome of the obligate intracellular parasite *T. gondii* encodes five putative FLIPs and four CDC50s. Here we report a genetic investigation to identify the localization and function of these uncharacterized family of proteins. Remarkably, FLIP1 and its cofactor CDC50.4 are responsible for the translocation of phosphatidylserine (PS) at the plasma membrane and ensure microneme exocytosis. In this context, *T. gondii* DOC2.1, which has been previously implicated in microneme secretion, binds to PS. Point mutations in the C2 domains of DOC2.1 are currently being generated to map the binding determinant to PS and its impact on microneme exocytosis. In an effort to identify partners of DOC2.1 that trigger membrane fusion, we investigated conserved and divergent SNAREs and three regulatory Sec1 or Munc18-like proteins (SM). Two of them appear to govern Golgi homeostasis, impacting on parasite growth and pellicle biogenesis.

162. Lost in translation. Understanding mitochondrial translation in *Toxoplasma*

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Abstract. How do you run a conveyor belt production line when the belt is cut into pieces and the workers are in another room? *Toxoplasma* must face this challenge to survive. The essential production of proteins in their mitochondrion, must take place with fragmented rRNA and with tRNAs imported from outside the mitochondrion. We identified unique parasite components and novel cellular structures that enable this essential operation. We demonstrate the essential role of *Toxoplasma* mitochondrial proteins in mitochondrial translation and parasite survival and make an innovative hypothesis about the mechanism for mitochondrial tRNA import in these parasites.

Funding. Funder acknowledgment: Royal Society of Edinburgh Biotechnology and Biological Sciences Research Council

SATURDAY 22 JUNE 2019

SESSION X - HOST PARASITE INTERACTIONS II**163. Discovery of Novel Bradyzoite Dense Granule Proteins in *Toxoplasma gondii***

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Abstract. During the acute infection, *Toxoplasma* tachyzoites invade their host cells where they form a parasitophorous vacuole (PV) that is necessary for intracellular survival. During the chronic infection, the parasites switch to slow-growing bradyzoites, and a hardy cyst forms around the PV allowing the parasite to persist for the life of the host. To maintain its intracellular niche and avoid host defenses, *T. gondii* secretes dense granule proteins (GRAs) into the PV space and cyst. Few bradyzoite-specific proteins have been discovered, and their roles in establishing and maintaining the chronic infection are largely unknown. To identify bradyzoite GRA candidates, we implemented the BioID approach using biotin ligase BirA* fused to MAG1, a bradyzoite-upregulated dense granule protein. To promote the switch to bradyzoites, the AP2IV-4 repressor of bradyzoite development was disrupted in the parasites. Using this approach, we identified 9 new GRAs that were confirmed by endogenous gene tagging and immunofluorescence. Two of these proteins were barely detectable in tachyzoites but readily visualized in the vacuole when the parasites were switched to bradyzoites. We functionally characterized several of these novel GRAs by gene deletion. Knockouts of two of these proteins demonstrated that they play important roles in parasite replication *in vitro*, and their absence results in lower cyst burden *in vivo*. Disruption of a third protein produced normally replicating tachyzoites but resulted in fewer cysts *in vivo*. This study helps to identify key proteins involved in maintenance of the chronic infection and promises to provide lead for developing novel therapeutics for toxoplasmosis.

Funding. This research was supported by the UCLA Molecular Pathogenesis Training Grant 5 T32 AI 7323-29 and NIH/NIAID R21 grant AI125106-01.

164. Identifying host proteins that are required for *Toxoplasma gondii* sequestration of host mitochondria using quantitative mass spectrometry

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Abstract. Host mitochondrial association (HMA) is a well-known phenomenon during *Toxoplasma gondii* infection of the host cell. The multicopy locus mitochondrial association factor 1 (MAF1) is required for HMA and MAF1 encodes distinct paralogs of secreted dense granule effector proteins, some of which mediate the HMA phenotype (MAF1b paralogs drive HMA, MAF1a paralogs do not). To identify host proteins required for MAF1b-mediated HMA, we performed quantitative proteomics on host cells infected with Type II parasites expressing MAF1b, MAF1a, and a C-terminal mutant of MAF1b that is also

HMA incompetent. Across all three samples we identified ~1,360 MAF1-interacting proteins, but only 12 that were uniquely and significantly enriched in MAF1b pulldowns compared to controls. The gene products include multiple host mitochondria outer membrane proteins, including proteins critical for mitochondrial protein import and immune modulation. Our work is now focused on characterizing each candidate's role in HMA using both siRNA knockdown and ectopic expression, and preliminary data show that knockdown of a mitochondrial import receptor decreases HMA efficiency during infection. These results show that the interface between the *T. gondii* vacuole and the host mitochondria is characterized by direct and/or indirect interactions between a single parasite effector and multiple target host proteins, some of which may be critical for the HMA phenotype itself. The elucidation of the functional members of this complex will permit us to explain the link between HMA and changes in the biology of the host cell and the inflammatory response.

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165. *Toxoplasma gondii* infection impairs myogenesis *in vitro*

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Abstract. *Toxoplasma gondii* is an intracellular parasite capable of crossing the transplacental barrier and infecting fetal tissues, causing congenital toxoplasmosis. The parasite has a strong tropism for the skeletal muscle cells, in which it forms tissue cysts. We investigated the molecular mechanisms that could interfere in the myogenic program during *T. gondii* infection. C2C12 myoblasts were infected with ME49 strain of *T. gondii* 24h after plating. 24h after infection proliferation medium was replaced for differentiation medium. At 24 or 120h after induction, cells were processed to immunofluorescence or qRT-PCR and conditioned medium was collected for cytokines/chemokines profiling using Cytokine Bead Array and ELISA. MMP activity was determined by zymography. Infection reduced the differentiation and fusion indexes, as well as the number of mature myotubes. The expression of myosin heavy chain was reduced in infected cultures. Expression of myogenic regulatory factors (MyoD, Myf5, Mrf4, myogenin) was also altered by infection. After 120 h of cultivation in differentiation medium, infected cells showed a significant increase of Ki67-positive cells, indicating increased proliferation. IL-6, MCP-1 and MMP-3 were highly increased in *T. gondii* infected cultures whereas TGF- β was decreased. In order to investigate upstream steps of the myogenic program, we looked for nuclear translocation of b-catenin. 10 hours after induction with differentiation medium, infected cultures showed decreased nuclear b-catenin staining. *T. gondii* induces a severe disarray of the SkMC, altering its secretory profile, leading to an unresponsiveness to Wnt/b-catenin pathway activation, which in turn induced the culture to remain in a proliferative, undifferentiated state.

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166. Mapping novel components of the *Toxoplasma* basal complex by BioID portrays an expanded hierarchy of its assembly

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Abstract. During its lytic cycle, *Toxoplasma gondii* replicates by an internal budding mechanism hereby forming two new parasites. Budding concludes with the actions of the contractile basal complex (BC). The BC molecular composition, its order of assembly and its mode of action are incompletely understood but differ substantially from final cytokinesis events described in other organisms. To understand this unusual cytokinesis apparatus, we have dissected its proteomic composition by proximity-dependent biotin identification (BioID). Hereto we fused several BC components with the small biotin ligase BioID2. Our results show that BioID2 is very suitable to reveal new BC components as indicated by the identification of novel proteins. Following their dynamics during endodyogeny, we assign novel components to BC initiation, its expansion and its mature phase. To further grasp the internal organization

of the BC we have assembled a protein-protein interaction network, based on interaction probability predictions, which mirrors our spatio-temporal observations. The phenotypic characterization of BC constituents highlights a critical function for components found in its initial assembly around the centrosome and during the final constriction phase at the end of cell division. In addition, our data indicate a third critical stage in between those two steps when nascent daughter cells are tapered. However, we have so far not identified single components that act in tapering, hinting that this process might rely on redundant factors. Our data set reveals a multilayered basal complex architecture and pictures a refined hierarchy of BC assembly; subsequently we characterize novel components critical for *Toxoplasma* division

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167. Quantitative visualization of an acute drop in parasitophorous vacuole pH immediately prior to *T. gondii* tachyzoite egress

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Abstract. The *T. gondii* lytic cycle is a repeating loop of host cell invasion, replication, egress, and re-invasion into the next host cell. Egress remains the least understood of these events. A perforin-like protein, PLP1, has been shown to be necessary for permobilizing the parasitophorous vacuole (PV) membrane for exit from the host cell. In vitro studies indicated that PLP1 activity is enhanced at low pH. This together with indirect evidence using superecliptic pHluorin suggested that a decrease in PV pH might facilitate egress. However, precisely when or to what extent PV pH changes during egress remained unknown. Using a ratiometric pHluorin that responds to changes in pH with changes in its bimodal excitation spectrum peaks, we directly measured the pH in the PV prior to and during induced egress. We observed a significant reduction in PV pH in both wild-type RH and Δ plp1 vacuoles. Interestingly, if parasites are paralyzed with cytochalasin D, a pH drop is still observed in RH but not in Δ plp1 tachyzoites. This indicates that the pH drop is independent of parasite motility, but reliant on PLP1 expression. We reasoned that one or more proton pumps could contribute to acidification of the PV during egress. There are two candidate P-type H⁺ ATPases termed Plasma Membrane ATPases 1 and 2 that are predicted to be expressed on the parasite surface. Ongoing work involves interrogating these pumps together with other potential players to determine the mechanistic basis for the decrease in pH observed during host cell egress.

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168. A versatile CRISPR screening platform for tailored in vitro and in vivo genetic screens identifies novel virulence factors in *Toxoplasma gondii*

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Abstract. Genome wide CRISPR screening is a powerful tool to identify genes required for survival in certain conditions. However, the inherent large scale of genome wide screens requires large amounts of starting material and importantly hampers *in vivo* analyses, where the animal would succumb to high infectious doses. To address host-pathogen interactions during *in vivo* *T. gondii* infection, a more versatile screening method was required. We have set up a tailored CRISPR screening system with arrayed gRNA for single step cloning of gRNA pools in to a custom CRISPR/Cas9 vector. This system allows the pool size, the genes targeted and the controls to be customised to the experimental question. We show reproducible cloning and *in vitro* screening with multiple library sizes and establish an analysis pipeline for small scale libraries. To test the system *in vivo*, we selected a pool size of 1000 gRNA to be compatible with mouse infections while providing sufficient coverage at the gRNA level. Assaying pooled *T. gondii* mutants through mice recapitulated expected phenotypes of known virulence factors, and importantly identified novel genes that are required for infection. Interestingly, some previously reported virulence factors are essential for *T. gondii* survival in the murine host while others appear to be non-essential if part of a larger pool, highlighting the complexities of host-pathogen interactions during infection

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169. An *in vivo* CRISPR/Cas9 screen identifies a novel *Toxoplasma* rhoptry protein that modulates *Toxoplasma* dissemination by affecting migration of dendritic cells

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Abstract. How *Toxoplasma* modulates the host immune system, overcomes *in vivo* nutrient deficiencies, and disseminates to distant organs is poorly understood. *Toxoplasma* co-opts the host cell by secreting proteins from specialized organelles called rhoptries (secreting ROPs) and dense granules (secreting GRAs). Most ROPs and GRAs have unknown functions. We used CRISPR/Cas9 and a single guide RNA library designed to target 217 *Toxoplasma* genes with which we created a pool of *Toxoplasma* loss-of-function (LOF) mutants, which we then used to infect mice and to study the effect of each gene on infectivity. We identified various genes that affected *Toxoplasma* fitness at the site of infection or dissemination and/or survival in distant organs. Because macrophages are one of the major cell types infected by *Toxoplasma in vivo* and because IFN γ is the major cytokine mediating resistance to *Toxoplasma*, we also performed genome-wide LOF screens *in vitro* to identify *Toxoplasma* genes required for fitness in IFN γ -stimulated and naïve macrophages. We further generated *Toxoplasma* single gene knockouts for some of our top hits, which were outcompeted by wild-type in *in vitro* and *in vivo* growth competition assays confirming the validity of the screens. For one of our hits we confirmed an *in vivo* dissemination defect and identified that it interacts with the host WAVE regulatory complex, which modulates the actin cytoskeleton through interaction with the Arp2/3 complex. *In vitro* data show that this *Toxoplasma* rhoptry protein is involved in modulating the actin cytoskeleton of dendritic cells thereby enhancing their motility and transmigration ability.

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170. A phenotypic screen to identify actin regulatory proteins

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Abstract. The acto-myosin system of apicomplexan parasites is critically involved in parasite gliding motility, host cell invasion and intracellular parasite development. With the adaptation of a chromobody in apicomplexan parasites it is now possible to study the highly complex regulation and dynamics of F-actin during the life cycle of these parasites. Surprisingly, *Toxoplasma gondii* forms an impressive intravacuolar F-actin network during intracellular parasite growth, consisting of short, highly dynamic F-actin. This intravacuolar network defines the so called residual body (RB), which acts as a recycling hub for maternal organelles. However, apicomplexans possess only a reduced set of (conserved) F-actin binding proteins, leading to the hypothesis that these diverged eukaryotes evolved unique, apicomplexan-specific actin regulatory proteins that cannot be identified via standard bioinformatic analysis. Given the importance of F-actin throughout the apicomplexan life cycle, we hypothesised that some of these proteins are essential. Based on the identification of critical genes in a recent, genome-wide screen (Sidik et al., 2016), we designed a curated library containing all gRNAs targeting essential genes conserved in apicomplexans. In order to perform a phenotypic screen on known phenotypes caused by disruption or stabilisation of F-actin, we adapted a conditional Cas9-system (splitCas9) and expressed the actin-chromobody as indicator. We identified several candidate genes, causing similar phenotypes upon their disruption and are currently localising the respective proteins and characterising their function in detail.

Funding. This work was supported by Wellcome Trust 087582/Z/08/Z Senior Fellowship and ERC Starting grant.

171. *Toxoplasma gondii* an Obligate Intracellular Protozoan Pathogen Relies on Its Own Heme Biosynthesis for Infection.

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Abstract. *Toxoplasma gondii* causes severe infectious disease in humans. *Toxoplasma* biosynthesizes and also scavenges small nutrient molecules from host cells to support its intracellular growth. Heme is an essential nutrient in all organisms, serving as a prosthetic group conjugated to many proteins for many fundamental subcellular activities. *Toxoplasma* encodes all the enzymes in the de novo heme biosynthesis pathway, suggesting that the parasites are able to self-supply heme. However, it remains unclear what is the extent to which *Toxoplasma* parasites rely on their heme biosynthesis pathway to support their intracellular growth. In this study, we genetically interrupted the parasite's de novo heme biosynthesis pathway by generating complete or conditional single-enzyme knockouts of 4 enzymes residing within the pathway. Disruption of these enzymes dramatically reduced the total heme contents in the parasites. These heme-deficient strains also exhibited strong growth defects, more strikingly, a total loss of acute virulence in a murine model, suggesting that the heme biosynthesis pathway plays a key role in the pathogenesis of toxoplasmosis. The addition of heme to the growth medium did not rescue the defects in plaque formation for the heme-deficient strains, which excludes the possibility that *Toxoplasma* can access extracellular free heme. In contrast, mammals can acquire heme from their dietary resources, suggesting that inhibition of heme production could specifically block heme acquisition in *Toxoplasma* parasites. In summary, our findings will shed light on the development of novel strategies to block heme production and acquisition in order to benefit clinical management of *Toxoplasma* infection.

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172. Old target new mechanism: Antifolate inhibitor from MMV collection acts through an apicoplast mediated delayed death mechanism in apicomplexan parasites

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Abstract. The MMV Box molecules are excellent starting points for discovering potent antiparasitic molecules with unique chemistry and novel mechanism of action. Phenotypic screening in *Toxoplasma gondii* identified 27 delayed death inhibitors, which were found to interfere with apicoplast segregation during daughter cell formation. To address their likely mechanism of action, we used chemical mutagenesis to generate mutant parasites resistant to 7 of these molecules, and identified the resistance conferring mutations by sequencing the genome of clonal isolates of the mutants and subsequent validation. Resistance to one of these molecules (MMV688345), which is a derivative of methotrexate, was due to a single amino acid change (F32L) in the DHFR-TS gene. Transgenic expression of this mutant DHFR-TS gene in wild type parasites was sufficient to confer resistance to MMV688345. Moreover, the DHFR-TSF32L mutant also conferred partial resistance to pyrimethamine. MMV688345 was found to have a delayed death effect in *Plasmodium falciparum* as well, which was fully rescued by IPP supplementation, thereby substantiating a similar mechanism of action for this inhibitor in the two parasites. The fact that inhibition of the DHFR-TS enzyme activity causes delayed death in apicomplexan parasites is intriguing and the probable mechanism of how this might happen will be presented.

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173. Global cysteine reactivity profiling in *Toxoplasma gondii* via chemical proteomics reveals new potential drug targets

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Abstract. Reactive cysteine residues display remarkable functional plasticity in proteins, mediating processes such as enzyme catalysis and being the target of post-translational modification. In the protozoan parasite *Toxoplasma gondii*, reactive cysteines are associated with proteins involved in host-cell invasion, gliding motility and organelle biogenesis. Despite this, a comprehensive understanding of the proteins dependent on such nucleophilic residues for their activity is lacking in *T. gondii*. Using a quantitative mass spectrometry approach, we profiled cysteine reactivity in extracellular *T. gondii* parasites and identified over 1000 proteins. Enrichment analyses revealed 130 hyperreactive sites in 97 proteins with diverse molecular function and localization. Intriguingly, we observed significant enrichment for essential genes, several of which encoding hyperreactive cysteines that are highly conserved amongst eukaryotic pathogens. To systematically assess the importance of these reactive sites on *T. gondii* biology, we have established a novel CRISPR-Cas9-based screen that enables assignment of amino acid function via a next generation sequencing readout. This study provides an initial insight into global thiol reactivity in the Apicomplexa and highlights novel targets/sites that could be exploited in covalent drug discovery.

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174. Simultaneous Inhibition of Both Cytochrome b Substrate Binding Sites is Synergistic against Experimental Toxoplasmosis

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Abstract. Endochin-like quinolones (ELQ) are highly effective compounds against the apicomplexan pathogens, *Toxoplasma gondii*, *Plasmodium falciparum* and *Babesia microti*. ELQs inhibit parasite proliferation by competing with the cytochrome bc1 complex substrates, ubiquinone and ubiquinol. Key structural features of ELQs determine whether ELQs inhibit the quinone reduction (Qi) site of cytochrome b or the quinol oxidation (Qo) site of cytochrome b, like atovaquone, a clinically used drug for toxoplasmosis. ELQ-316 is a lead preclinical compound that binds to the Qi site. We tested the combination of ELQ-316 and atovaquone as well as ELQ-316 and ELQ-400, a Qo targeting ELQ, against *T. gondii* and found synergistic inhibition in a 72-hour proliferation assay. The synergistic effect was also observed 72 hours after the replacement of media without inhibitors. To test synergy *in vivo*, ELQ-422, an alkoxy carbonate ester prodrug form of ELQ-316 with improved bioavailability, was combined with atovaquone against an RH strain *T. gondii* infection in mice. The combination of ELQ-422 and atovaquone resulted in survival of all mice compared to 50% survival in an equivalent dose of ELQ-422 and no survival in an equivalent dose of atovaquone. Ongoing experiments are focused on determining the mechanism of synergy from combining Qo and Qi site inhibition. The synergistic drug combination of ELQ-316 and atovaquone, a well-tolerated clinically used drug, could potentially provide a safer, more effective treatment for toxoplasmosis.

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175. Identification of a Potential Itraconazole Target in *T. gondii*

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Abstract. Itraconazole, a drug used clinically to treat fungal infections, has low-nanomolar activity against *Toxoplasma gondii* in vitro. In fungi, itraconazole inhibits the cytochrome P450 lanosterol 14 α -demethylase. The target in *T. gondii* has not been identified. Fluorescently labeled posaconazole, a compound with structural similarities to itraconazole, localizes to the *T. gondii* mitochondrion. We generated itraconazole-resistant clonal strains through chemical mutagenesis, clonal selection at 10x itraconazole IC50 and comparison of the whole genome sequences of the resistant and parental clones. Nonsynonymous SNVs were found in 14 genes. Only one of these, TGGT1_281440, conferred a significant loss of fitness in the genome-wide loss of function screen performed by Sidik et al. Repeat mutagenesis and selection resulted in 5 more resistant clones: 2 clones with the same V3414E substitution as the original resistant clone, one clone with a nearby M3412K mutation and 2 clones in which a mutation is yet to be identified. TGGT1_281440 is predicted to encode a 420kD transmembrane protein of unknown function. Analysis of the secondary structure of the protein predicts the formation of a beta-barrel in the C-terminal region. A similar beta-barrel structure is also found in an itraconazole target that has been characterized in human umbilical vein endothelial cells, voltage-dependent anion channel 1 (VDAC1). These results indicate that itraconazole interacts with a novel target in *T. gondii* and have important implications for future optimization of triazoles for toxoplasmosis.

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176. Characterisation of a Candidate Protein of the *Toxoplasma gondii* Mitochondrial ATP Synthase Complex

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Abstract. The mitochondrial ATP synthase complex utilises the proton gradient established by the electron transport chain to drive ATP synthesis. This is achieved via the flow of protons through a membrane bound molecular rotor in ATP synthase called F₀, which is coupled to ADP phosphorylation at the F₁ component. The genomes of most apicomplexan parasites, including *Toxoplasma gondii*, the causative agent of toxoplasmosis, encode canonical subunits of the F₁ domain. However, proteins from the F₀ domain are not clearly identifiable. Recent proteomic studies of *T. gondii* mitochondria have identified novel candidate proteins of ATP synthase in these parasites. One of these proteins, termed *T. gondii* ATP Synthase Associated Protein-1 (TgASAP-1), is conserved in apicomplexan parasites and closely related organisms. Here, we demonstrate that TgASAP-1 is an integral membrane protein of the mitochondrial ATP synthase complex of *T. gondii*. Conditional knockdown of TgASAP-1 impairs parasite growth, resulting in incomplete assembly of the ATP synthase complex and defects in mitochondrial respiration. A conserved arginine residue in TgASAP-1 is important for its function, suggesting that TgASAP-1 may be the previously unidentified subunit a of the F₀ rotary motor in *T. gondii*. Our findings highlight the importance of the ATP synthase complex *T. gondii* mitochondrion and expands work on the novel biology of the parasite's mitochondrion.

Funding. ANU

177. CDP-ethanolamine pathway for PtdEtn biogenesis is required for sphingolipid synthesis

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Abstract. Phosphatidylethanolamine (PtdEtn) is the second major class of lipids in apicomplexan parasite *Toxoplasma gondii*. The synthesis of PtdEtn can be achieved via two routes: decarboxylation of phosphatidylserine (PtdSer) by

phosphatidylserine decarboxylase (PSD), or de novo synthesis involving three reactions termed Kennedy pathway. The PSD-dependent biogenesis of PtdEtn has been described in tachyzoites; however, the physiological relevance of PtdEtn contributed by Kennedy pathway has not been clear. We identified an ethanolamine cytidyltransferase (TgECT), the rate-limiting enzyme of Kennedy pathway, which is localized in the cytoplasm. The enzyme is clearly essential for the lytic cycle as its genetic ablation is not feasible, and auxin-mediated conditional knockdown severely impairs the parasite growth in plaque assays. Lipidomic analysis of the mutant identified a role in regulation of the synthesis of multi-classes phospholipid. Moreover, we discovered that TgECT is required for the generation of ceramide-phosphoethanolamine (CPE), a rare sphingolipid present only a limited number of organisms and absent in mammals. Our further work indicated the presence of an insect-like CPE synthase in the parasite that is currently being investigated.

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POSTERS PRESENTATION**POSTER SESSION I****201. Analysis of treatment effect in patients with ocular toxoplasmosis in Quindío; Colombia**

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Abstract. The purpose of this study was to describe the effect of treatment on the clinical outcomes of patients with ocular toxoplasmosis in one referral center in Quindío, Colombia. We analyzed retrospectively clinical records of patients attending between 2014 to 2018. Patients with active retinochoroiditis lesions, anti-*Toxoplasma gondii* IgG positive in serum and a minimal follow-up of 6 months, were included. Twenty-nine patients were included in the analysis. The median age was 26 years. The median of lesions was 2 and of the size of lesions was 1 disc diameter (DD). The schemes used were sulfadoxine pyrimethamine in 58.62%, trimethoprim sulfamethoxazole in 27.59%, clindamycin in 24.14% and azithromycin in 10.34%. The 35.71% received antibiotics alone and 57.14% antibiotics plus steroids. The median of inflammation resolution was 33 days, the improvement of the visual acuity (VA) was one line and the median on percentage of lesion size reduction was of 50%. There was a statistically significant relationship between greater number of days of inflammation and use of clindamycin (p=0.010). The concomitant use of steroids (p=0.050) and non-steroidal anti-inflammatory drugs (p=0.030) was associated with fewer days of inflammation. The improvement in VA showed a statistically significant relationship with the use of antibiotics plus steroid (p=0.050) and the use of NSAIDs (p=0.011). In conclusion, in this series of cases, the use of clindamycin was associated with worse outcomes in the resolution of inflammation. Positive outcomes were evidenced with the use of steroid plus antibiotics and NSAIDs in this group of patients.

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202. Current situation of the management of toxoplasmosis during pregnancy in Armenia (Colombia)

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Abstract. In 2013 the Colombian official guidelines for management of toxoplasmosis during pregnancy were launched. Here we reviewed how these guidelines has been implemented and which has been its impact. We reviewed the clinical charts of Redsalud, the largest public center of health service in Armenia, Quindío. Between 1809 pregnant women attended during 2018, 26 had the diagnosis of gestational toxoplasmosis (1,43% IC 95% 1,71-1,1). Serological tests were requested at a mean of 11,9 weeks of gestation (range: 7-27) which coincided with the week where pregnant women arrived to its first prenatal consultation. Results of serological tests were delivered in mean at week 19 (range 8,5 – 32,4). In 23% (n: 6) diagnosis was made during the first 16 weeks of gestation, all these women had an avidity test, three of them had a result of high avidity and prenatal treatment was stopped after the result. In 57,6% of pregnant women (n: 15) diagnosis was after week 16 of gestation, all

women had an IgA test as recommended by the guidelines, one of them was positive, in all cases treatment was continued. In 19% of cases (n=5) diagnosis was made by seroconversion criteria after monthly serological follow up with IgM. Three amniocentesis were performed and results were negative. There were not echography anomalies in this cohort. This study shows that in Armenia at the public health service there exist a good implementation of guidelines. The delivery of results should be improved.

Funding. Universidad del Quindío, GEPAMOL.

203. CXXX motif of *Toxoplasma gondii* Hsp40 TgJ1 is required for pathogenesis

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Abstract. *Toxoplasma gondii* has a type I Hsp40 of DNAJA group that is highly similar to cytosolic yeast Ydj1 or human Hdj2, (TGGT1_311240, TgJ1). These Hsp40s have a C-terminal CXXX motif which is able to be farnesylated, a PTM that allows association with the endoplasmic reticulum and the mitochondrial surface. TgJ1 is cytosolic in normal culture conditions but translocates to the nucleus during heat shock treatment and pH8.1 stress. TgJ1 gene is essential. A stable *T. gondii* line that expresses TgJ1 without the CXXX (CRQQ) motif (TgJ1ΔCXXX) was generated in RH strain using a CRISPR/Cas9 strategy. TgJ1ΔCXXX is found in the cytosol and nucleus in normal conditions. TgJ1ΔCXXX parasites exhibit a fitness defect of the lytic cycle and has a high rate of bradyzoite differentiation under alkaline stress. TgJ1ΔCXXX parasites also show an increase in the level of phosphorylated eIF2- α in normal growth conditions, likely due to endoplasmic reticulum stress from unfolded proteins. BALB/c mice infected with 100 tachyzoites of the parental (RH strain) died between 8-12 days post-infection whereas mice infected with same doses of TgJ1ΔCXXX parasites survive. Re-infection of mice infected with TgJ1ΔCXXX 30 days postinfection with RH strain also survived suggesting that inoculation of TgJ1ΔCXXX tachyzoites generated a complete immunity against *T. gondii*. Co-IP and mass spectrometry analysis indicated that TgJ1 interacts with a large number of proteins involved in different biological processes and subcellular localization. Mutant TgJ1 is defective at interacting with these proteins, especially with those associated to membranes and rhoptry localization.

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204. Development of a SAG1-based multiplex assay for large-scale seroepidemiological surveys of IgG responses to *Toxoplasma gondii*

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Abstract. A few studies have reported the implementation and application of bead-based multiplex assays for seroepidemiological surveys regarding *Toxoplasma gondii* infection. They come in different flavors and each has specific limitations. Here we report the expression and use of full-length, soluble and *in vivo* biotinylated recombinant SAG1 and SAG2A for the detection of anti-*T. gondii* IgG antibodies. It allowed an oriented, reproducible coupling to magnetic Lumindex beads, requiring only minute amounts of protein per determination. We validated our assay with more than 1.300 human sera previously tested with the Vidas TOXO IgG II and found very high concordance with this commercial assay regarding both, sensitivity and specificity (ROC >0.99), even when only biotinylated SAG1 was used as sole antigen.

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205. Did maritime trade between Europe and West Africa influence *T. gondii* genetic diversity?

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Abstract. Exploring the population structure of *Toxoplasma gondii* is crucial to understand its worldwide distribution and the determinants of its evolution. In Africa, its spatial structure is still non-uniformly studied and only few articles reported strain genetic diversity in fauna and disseminated toxoplasmosis in African diaspora. Our IntroTox project investigates the possible role of past and present maritime trades on *T. gondii* introgressions between France and West and Central Africa, two regions of the world sharing a long commercial history since colonialism. We present here results of a study conducted in Benin, in comparison to previous work in Senegal and Gabon. Thirty-nine genotypes were obtained from two littoral cities (Cotonou and Ouidah) and two inland cities (Parakou and Natitingou). Genotyping using 15 microsatellite markers revealed a predominance of the autochthonous African lineage Africa 1 (36/39), highly virulent in laboratory mice. The remaining genotypes were one Africa 4 (1/39) and clones of Type III (2/39). Considering its wide distribution, Africa 1 seems to be the equivalent of European Type II in the African continent. This predominance of autochthonous strains is similar to what has been reported in Gabon (Mercier et al., 2010), where, however, Africa 1, Type III and Africa 3 were found in equal proportions. The population structure tends to refute a possible role of maritime trade in Benin, in opposition to Senegal (Galal et al., 2019), despite the fact that Benin was one of the most important hubs at the time of the slave trade in Africa.

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206. Effect of ROP16 and ROP18 proteins of *T. gondii* on individuals with toxoplasmosis

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Abstract. *T. gondii* ROP16 and ROP18 proteins have been identified as important virulence factors for the parasite. Here, we describe the effect of ROP16/ROP18 proteins in peripheral blood mononuclear cells (PBMC) from individuals with different clinical manifestation. To achieve this, we had evaluated the INF- γ , IL-10, and IL-1 β secretion levels in supernatants of PBMC from ocular toxoplasmosis (n=12), chronic asymptomatic (n=8) and *T. gondii* seronegative (n=10) individuals infected with tachyzoites of RH and null mutants (RHΔrop16 and RHΔrop18) strains and to determine if the production of these cytokines may be influenced by the polymorphisms of host immune related genes. We found that individuals with ocular toxoplasmosis produce less INF- γ than chronic asymptomatic individuals but it is ROP16/ROP18 independent. Additionally, although it is clear that ROP16 protein phosphorylates host STAT3/6, which resulting in limiting the protective Th1 cytokine response; in our study, the phosphorylation of the transcription factors STAT3/6 after RH and RHΔrop16 *T. gondii* infection did not present a significant difference between OT and Asym individuals. This suggests that *T. gondii* ROP16 protein is not the only kinase that phosphorylates STAT3/6 in PBMCs of individuals previously infected with this parasite. Finally, we found polymorphisms that influence the cytokine profile in pro-inflammatory cytokines such as INF- γ , and IL-1 β that could enhance susceptibility to ocular toxoplasmosis in *T. gondii* infection.

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207. Evaluation of RNA extraction method; Detection of RT-qPCR from Oocysts of *Toxoplasma gondii* and its Application in Field Samples

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Abstract. Introduction. Infections by waterborne protozoan are one of the most common causes of disease in humans worldwide. *Toxoplasma gondii* is highly resistant to chemical disinfectants. The current method used to determine the viability of these oocysts in the laboratory is the bioassay. However, it is expensive and only provides qualitative evaluations. Therefore, new affordable methods for this monitoring in drinking water samples are necessary. Materials and methods. We evaluate different protocols for RNA extraction. The limit of detection of RT-qPCR was determined by dilutions of the protozoan RNA evaluated. The specificity assays were performed using RNA from different protozoa. Finally, 25 samples taken before, during and after the treatment plant were evaluated by optical microscopy and conventional PCR with standardized conditions. Results The best method of extracting RNA from oocysts of *Toxoplasma gondii* was mechanical lysis. The limit of detection by RT-qPCR was 2 parasitic forms with a CT: 35.4 CT SD: 0.27. The applicability was evaluated in drinkable water taken at a treatment plant in the department of Quindío and were inoculated with viable oocysts from 500 to 2 oocysts. Conclusions: RNA assays performed were specific and inexpensive. The evaluation of the method demonstrated its ability to detect viable oocysts of *Toxoplasma gondii* at low concentrations. RT-qPCR assays are useful to evaluate and monitor the viability of *Toxoplasma gondii* oocyst in water samples.

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208. Expression of inhibitory receptors on CD8+ T cells from ocular toxoplasmosis individuals

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Abstract. Chronic toxoplasmosis is usually asymptomatic in humans. One of the clinical manifestations of *T. gondii* is ocular toxoplasmosis (OT), which can lead to uveitis and visual disability. Several parasite and host factors that may explain the development of symptoms have been suggested. In a murine model of chronic toxoplasmosis, a phenotype that is consistent with T CD8 cell exhaustion has been reported. In this work, we evaluated the expression of 4 exhaustion markers (PD-1, CD244, LAG-3 and CD160) in PBMCs from individuals with OT whom have different ocular scars numbers compared with asymptomatic and seronegative individuals as a control group. The presence and level of expression of exhaustion markers was determined in CD3+ and CD8+ lymphocytes by flow cytometry. We compared the percentage of positive cells and mean fluorescence intensity for each exhaustion marker. In accordance with our hypothesis, we found more ARNm levels of PD-1 and CD244 and slightly increased MFI in the same markers in OT individuals. Our preliminary results suggest that these individuals show a partial exhaustion phenotype because of PD-1 and CD244 increased levels in individuals with more ocular scars.

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209. First report of *Toxoplasma gondii* in bats in Colombia

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Abstract. *Toxoplasma* infections have been reported in a large number

of warm blooded animals around the globe, but infections in bats have been scarcely reported. In Colombia, there are not reports about *T. gondii* presence or infection in bats; according to that, the objective of the present study was to report the detection of *T. gondii* DNA from internal organs from bats in Quindío, Colombia. Three *Carollia brevicauda* bats were captured and euthanized in the Natural Reserve "La Montaña del Ocaso", Quimbaya, Quindío, Colombia. Lungs, heart, liver, kidneys, stomach, small and large intestine from each bat were extracted and stored at -20°C. Approximately 50 milligrams from each organ were cut and used for DNA extraction. Later, by nested PCR for B1 sequences was done to detect *T. gondii* DNA. The positive samples were sequenced to confirm by BLAST. In the present study, the kidney from one bat resulted B1 sequence PCR positive for *T. gondii* and it was confirmed by triplicate and sequencing. Our methodology was useful to detect *T. gondii* in organs from captured and euthanized bats. This is the first report of *T. gondii* DNA detection in Colombia and the second country, after Brazil, in South America.

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210. Food-borne risk (meat and vegetables) of *Toxoplasma* infection in Ibagué, Colombia

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Abstract. The FAO has ranked *Toxoplasma* fourth in its list of food-borne parasites to be prioritised for control, given its importance for global human health. Although some reports have dealt with it being found in meat in some Colombian cities, no studies have been concerned with its prevalence in vegetables and fruits destined for human consumption. We used nested PCR for analysing 186 samples of meat (62 beef, 62 chicken and 62 pork) and 100 samples of vegetables and fruit (15 coriander, 15 smooth lettuce, 14 carrots, 14 crisp lettuce, 14 strawberries, 14 celery and 14 scallions) purchased from retailers of Ibagué Colombia. It revealed 22.5% (14/62) positivity in pork, 19.3% (12/62) in beef and 14.5% (9/62) in chicken. In vegetables and in fruits 19%; 40,00% (6/15) in coriander, 28,5% (4/14) in strawberries, 26,6% (4/15) in smooth lettuce 21,4% (4/14) in celery, 21,4% (4/14) in scallions, 14,2% (2/14) in crisp lettuce and 0% in carrots (0/14). We have thus demonstrated *Toxoplasma* contamination in meat and vegetables sold for human consumption in Ibagué in Colombia.

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211. Genes from innate and adaptive immune responses in Ocular Toxoplasmosis

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Abstract. As the immune system modulates the response to infectious diseases including toxoplasmosis, we investigated innate and adaptive immune response genes as potential predictors for Ocular Toxoplasmosis (OT). Patients with OT (primary and recurrence) and controls, both with positive serology were recruited. The presumed diagnostic of OT was done by indirect binocular ophthalmoscope analysis. *T. gondii* serology (IgM, IgG) were assessed by ELISA. The HLA, KIR, and MICA alleles were identified by PCR-SSOP and the CCR5 polymorphisms were identified by PCR-RFLP. The cytokine mRNA levels were determined by qPCR. The chi-square, the exact's Fisher test, the OR value (CI 95%) and the p-value (>0.05) were used to compare the proportion among the groups. The MICA*002~HLA-B*35 and the MICA*008~HLA-C*07 haplotypes lost their association with high and low risk for OT, respectively, after adjusting the data for multiple comparisons. The KIR alleles their HLA ligands (KIR3DS1-Bw4-80Ile and KIR2DS1+/C2++ KIR3DS1+/Bw4-80Ile+) were associated with increased susceptibility for OT. The KIR-HLA inhibitory pairs -KIR2DL3/2DL3-C1/C1 and KIR2DL3/2DL3-C1- were associated with decreased susceptibility for OT while the KIR3DS1~/KIR3DL1+/Bw4-80Ile+ combination

was associated as a protective factor for OT, particularly against recurrent manifestations. CCR5/CCR5 genotype and simultaneously the CCR5-59029 AA or AG genotypes have a greater risk of developing OT. mRNA levels for TNF- α and IL-12 were up-regulated in OT patients. Genes involved in different steps of innate and adaptive immune responses against *T. gondii* infection influence the susceptibility or resistance for the development of OT.

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212. Identification of factors associated with recurrences in patients with ocular toxoplasmosis in Quindío; Colombia

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Abstract. The aim of this work was to identify the sociodemographic, clinical and epidemiological factors associated with recurrences in ocular toxoplasmosis. We reviewed retrospectively the clinical records of patients who consulted in the Health Center of the University of Quindío between 2004-2017. Patients with ocular lesions of retinochoroiditis that were anti-*Toxoplasma gondii* IgG positive and were followed for at least 12 months, were included. The recurrence rate was estimated through an adjusted recurrence index (IR) as follows: number of recurrences/months of follow-up \times 100. Kruskal-Wallis test, ANOVA, linear regression and Spearman coefficient tests were applied by using the software Epi Info 7 and SPSS 14.0. Statistically significant differences were considered if $p \leq 0.05$. A total of 77 patients were included, with a median of 23 years (range 0.7-61 years). 59.7% were women and 40.3% men. We found a higher IR associated with lowest socioeconomic level ($p = 0.03$) and with congenital infection ($p = 0.05$). Also, the congenital infection had a higher number of retinal scars ($p = 0.01$). The consumption of boiled water was related to a lower IR ($p = 0.0007$). There was a negative Spearman correlation ($r_2 = -0.41$, $p = 0.000$) between age and IR. In conclusion sociodemographic factors such as age and low socioeconomic status are associated with a high recurrence rate, as well as the congenital origin of infection. On the contrary, the consumption of boiled water was related to a lower rate recurrence of ocular toxoplasmosis.

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213. Immune responses of intestinal organoids from wild rodents upon infection with *Toxoplasma gondii*

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Abstract. The main route of infection of *Toxoplasma gondii* is orally through the intestinal epithelium. However there is lacking knowledge on the mechanisms by which the parasites invade intestinal epithelial cells and what the innate cellular responses to invasion are. Laboratory inbred mice have been mostly used for studying *T. gondii* infection mechanisms; however, their value for generalizing these findings to other rodents is unclear. For example, previous reports have shown that some wild *Mus musculus* species are resistant to virulent *T. gondii* strains due to the variability of immunity-related GTPase (IRG) genes, which was lost in laboratory mouse strains. Consequently, there is a need to verify these findings in other rodents which are relevant for the transmission mode to cats. For this reason, we have established protocols for the generation, cultivation and differentiation of small intestinal organoids from the vole *Myodes glareolus*. This rodent has been shown, among other wild rodent species, to be a more relevant prey to cats in Europe than *M. musculus*. We developed a protocol in which our organoids present an inverted polarity, allowing easier access of the parasite to the apical side of the intestinal epithelial cells. We have infected the organoids with different *T. gondii* strains and compared the infection in wild rodent organoids to those of laboratory mice.

Data of this comparison and the effect of IFN γ on parasite replication in this culture system will be presented.

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214. Oral Vaccines: Use Of Plants For The Expression Of The Main Surface Antigen 1 (Sag1) Of *Toxoplasma gondii*

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Abstract. There is an urgent need to develop vaccines against *Toxoplasma gondii*. Since the portal of entry of *T. gondii* is the mucosa, *Toxoplasma* infection is an interesting model to study the optimization of expression systems based on plants and to assess the effectiveness of oral plant-made vaccines. Here, in order to improve the expression levels of vaccine antigen expressed in plants, we evaluated the role of plant Hsp90s as carrier of the SAG1 antigen. We assayed stable transformation system of lettuce plants and transient expression of tobacco leaves by agroinfiltration to test the performance of four different constructs carrying mature SAG1 protein (SAG1m) or a B- and T-cell epitopes of SAG1 peptide fused to *Nicotiana benthamiana* Hsp90 (NbHsp90.3) or *Arabidopsis thaliana* Hsp90 (AtHsp81.2). The agroinfiltration with AtHsp81.2-SAG1HC construct produced the highest level of SAG1 expression being the AtHsp81.2-SAG1HC protein accumulation 60-fold higher than AtHsp81.2-SAG1m (120 μ g of AtHsp81.2-SAG1HC vs. 2 μ g of AtHsp81.2-SAG1m per gram of fresh leaves). On the other hand, SAG1m yields in transgenic lettuce plants expressing AtHsp81.2-SAG1m or NbHsp90.3-SAG1m ranged from 35 to 45 μ g per g of lyophilized leaves. Finally, a total of 5 positive human serum samples were able to react with the recombinant fusion proteins suggesting that the SAG1 versions fused to plant Hsp90s can be recognized by antibodies from *Toxoplasma*-infected humans. Our results suggest that plant Hsp90s improve the recombinant protein yields in plants and have the potential to elicit immune responses in immunization protocols.

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215. Population genetics and virulence of *Toxoplasma gondii*

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Abstract. *Toxoplasma gondii* is genetically diverse. There are only a few genotypes of *T. gondii* dominate in the northern hemisphere, however, many genotypes co-exist in South America and there is no clear dominance of any genotypes. In addition, *T. gondii* strains from South America are more likely to be virulent in mice. To understand what factors may have influenced modern-day genetic diversity and virulence of *T. gondii*, we conducted an integrated analysis of population genetics, ecology and virulence of *T. gondii*. The results suggest that the rise and expansion of farming in the past 11,000 years established the domestic cat-mouse transmission cycle for *T. gondii*, which has played a significant role in the selection of certain lineages of *T. gondii*. The analysis of the global type II lineage of *T. gondii* suggests its Old World origin but recent expansion in North America, which is likely the consequence of global human migration and trading. The allele combinations of polymorphic ROP18 and ROP5 genes are strongly associated with parasite virulence in laboratory mice. Mathematical simulations showed that within the domestic transmission cycle, intermediately mouse-virulent *T. gondii* genotypes have an adaptive advantage and eventually become dominant. Taken together, our study indicated that human played an important role on transmission of *T. gondii*.

216. Prevalence of *Toxoplasma gondii* by PCR in domestic cats stools (*Felis silvestris catus*) in Armenia; Quindío (Colombia) and genetic analysis of ROP18

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Abstract. Domestic cats (*Felis silvestris catus*) and other felines are the unique definitive hosts of *Toxoplasma gondii*, with the ability to excrete oocysts in the environment. The goal of this work was to determine the prevalence of *T. gondii* by conventional PCR and to analyze phylogenetically ROP18 sequences

from positive samples in domestic cats in the city of Armenia, Quindío. One-hundred and forty fecal samples from domestic cats were collected from 10 districts around the city. The samples were concentrated using Ritchie method and the DNA was extracted. Nested PCR for B1 sequence and ROP18 were carried out. We used MAFFT v7, jModelTest v2.1.1, Bayesian analysis by BEAST 1.8.3, Tracer v1.6, SplitsTree4 and PopArt for the ROP18 sequence and phylogenetic analysis. We found that 17.8% (25/140) B1 and 4.2% (6/140) ROP18 were PCR positive. Phylogenetic analyses showed that sequences were joined in a unique group closer to the archetypal type I of strains. In conclusion, through the nested PCR technique it was possible to identify the presence of *T. gondii* DNA of cat population indicating a great potential for spreading of this parasite in the city. These findings help to explain the high prevalence found in the human population of this city.

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217. Seroprevalence of *Toxoplasma gondii* in domestic pigs; sheep; cattle; wild boars; and moose in the Nordic-Baltic region: systematic review and meta-analysis

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Abstract. One of the ways humans may become infected with *Toxoplasma gondii* is if they consume undercooked meat of infected animals. We conducted a systematic review and meta-analysis of *T. gondii* seroprevalence in pigs, sheep, cattle, wild boars and moose in the Nordic-Baltic region, including studies from January 1990 to June 2018. Thirty-two studies qualified for meta-analysis: 13 on domestic pigs, 6 on sheep, 3 on cattle, 6 on wild boars, and 4 on moose. For each host species, we estimated the pooled apparent seroprevalence using a random effects model, and subgroup analyses were performed using mixed-effects models. The estimated pooled seroprevalence was 6% in pigs (CI95%: 3–10%), 23% in sheep (CI95%: 12–36%), 7% in cattle (CI95%: 1–21%), 33% in wild boars (CI95%: 26–41%), and 16% in moose (CI95%: 10–23%). In all host species except wild boars, the pooled seroprevalence estimate was higher in >1-year-old than in younger animals. The results indicate widespread exposure to *T. gondii* among animals raised or hunted for human consumption in the region.

Funding. The Nordic Source Attribution of Toxoplasmosis Working Group has been financially supported by the Nordic Council of Ministers. In addition, this work was partly supported by the Innovation Fund Denmark (7038-00129B), SEGES pig research center, the European Regional Development Fund "Post-doctoral research aid" project "One Health multidisciplinary approaches for epidemiology and prevention of selected parasitic zoonosis" (OMEPPAZ) (1.1.1.2/VIAA/1/16/204), and COST Action FA1408 "A European Network for Foodborne Parasites: Euro-FBP".

218. The importance of official protocols for toxoplasmosis - report of loss of follow-up in children in Brazilian teaching hospital

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Abstract. Protocols for diagnosis and treatment of gestational and congenital toxoplasmosis were established after the biggest worldwide outbreak in 2018, in Brazil. Before that, there was no official countrywide rule/protocol describing laboratory and clinical procedures for toxoplasmosis. Here we describe

the clinical findings in gestational and congenital toxoplasmosis. These retrospective study analysed 49 medical records of pregnant women who received prenatal monitoring at a teaching hospital. Data recorded as gestational age, treatment, clinical and laboratory findings. 39 records of their potentially infected newborns were screened observing: neurological, visual and otologic development and exams, prematurity and recommended treatment. The average age of the 49 pregnant women was $23,6 \pm 6,3$ (min:13; max: 39; median: 23); 22,4% (n=11) were primigravidae and 42,8% (n=21) multigravidae.; 75,5% (n=37) of the pregnant presented positive serology; 46,9% (n=23) underwent amniocentesis, 20,4% (n=10) had a positive amniotic fluid PCR and 8,16% (n=4) fetal ultrasound scans showed changes. Treatment included spiramycin or the triple scheme (sulfadiazine, pyrimethamine and folic acid). Among the babies were presented: positive IgM 2,5% (n=1), or positive blood PCR 7,69% (n=3) or suspicion and signs of clinical changes 17,94% (n=7); 17,94% (n=7) of them underwent treatment for congenital toxoplasmosis, based on the triple scheme, not necessarily those with clinical features. In conclusion, gestational toxoplasmosis is occurring in multigravidae more than in primigravidae; the positive PCR in AF confirm a reasonable number of fetal infection. The number of children underwent the treatment was very low even receiving health care in a teaching hospital.

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219. *Toxoplasma gondii* specific epitopes selected by bioinformatics tools elicit activation of cytotoxic activity in CD8+ T cells from individuals with toxoplasmosis

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Abstract. Identification of immunogenic peptides derived from *Toxoplasma gondii* proteins and its evaluation as potential activators of the cytotoxic immune response is one of the first steps for development of a subunit vaccine against toxoplasmosis. Therefore, we evaluated 4 markers of cytotoxic activity in memory CD8+ T-lymphocytes from individuals with toxoplasmosis stimulated with 2 peptides derived from *Toxoplasma* Got1 and Ter1p proteins selected by a bioinformatics pipeline in a previous work. We modeled the interaction of Got1-FLDRALLTL and Ter1p-FLADLLHSV with the HLA-A02 in order to have a scaffold for molecular dynamics simulations (MDS). Then, we selected HLA-A02 individuals seropositive for *Toxoplasma* by PCR-SSP. Flow cytometry (FC) was used to analyze the cytotoxic markers CD107a/CD107b, granzyme B and perforin in CD3+/CD8+/CD45RO+ lymphocytes from HLA-A02 positive individuals after stimulation with Got1 and Ter1p peptides. MDS using peptides-HLA-A02 complexes showed stable interactions, same as the positive control (GRA3-HLA-A02 complexed) showing that the interaction could elicit a cytotoxic response. FC assays showed higher levels of CD107a (p0.05). Summarizing, the peptides induced a higher expression of CD107a in memory T lymphocytes from individuals with toxoplasmosis, indicating a possible activation of a specific cytotoxic response, however an exhaustive evaluation is required in a humanized animal models to support this conclusion.

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220. *Toxoplasma* restriction in immune-stimulated primary human cells

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Abstract. The parasitophorous vacuole (PV) serves as the 'transaction interface' between *Toxoplasma* and host. It has been shown that there are differences in these 'transactions' dependent upon the host species, the parasite strain and cell type. Here, we focus on human cells and specifically primary endothelial cells (HUVEC). *Toxoplasma* strains actively invade and proliferate

within these cells with a proportion of parasites being eliminated in an acidified vacuole after immune stimulation of the cells by interferon gamma (IFN). This IFN-dependent killing partly relies on recruitment of host effectors to the PV, a process which appears to be initiated by Lys63-linked ubiquitin. We have identified an ankyrin repeat domain protein by SILAC which localises to the PV and is involved in parasite restriction. We are investigating interaction partners by APEX-tagging recruited proteins and establishing their significance using a high-throughput automated assay to determine parasite clearance and replication in order to establish a mechanism for parasite killing.

Funding. This work was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001076), the UK Medical Research Council (FC001076), and the Wellcome Trust (FC001076). EMF was supported by a Wellcome Trust Career Development Fellowship (091664/B/10/Z). DF was supported by a Boehringer Ingelheim Fonds PhD fellowship.

221. Toxoplasmosis in brown hares - one of the possible causes of death and population decrease in Czech Republic

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Abstract. In the present study, 36 brown hares (*Lepus europaeus*) originated from Southern Moravia regions of the Czech Republic were submitted for post mortem examination. Heart, lungs, liver, spleen, kidneys and bone marrow tissues were tested for *Toxoplasma gondii* using real-time PCR targeting 529 bp repeat element of the parasite. PCR positive samples were genotyped by single multiplex PCR assay with 15 microsatellite markers. All tissue samples were simultaneously used for cultivation of *Francisella tularensis* and *Brucella melitensis* as a part of national monitoring of tularemia and brucellosis. *T. gondii* was detected in two hares. In first hare, gross pathology showed pulmonary congestion, interstitial edema and alveolar emphysema, multiply miliary granulomas in liver and acute splenic tumor. DNA of *T. gondii* was demonstrated in lungs, heart, liver, spleen and kidneys. In the second hare, main macroscopical changes were pulmonary congestion, enlarged liver with focal necrosis and acute splenic tumor. *T. gondii* DNA was detected only in heart and spleen. Microsatellite genotyping revealed that both animals were infected by genotype II, considered not virulent for mice and endemic for European countries. *F. tularensis* was proved by culture technique in tissues of three hares. There was no coinfection of *T. gondii* and *F. tularensis*. All hares were negative for *B. melitensis*. Toxoplasmosis was found as a cause of death in two brown hares and therefore should be concerned with long term decrease of brown hares population in certain regions of the Czech Republic.

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222. Toxoplasmosis in French Guiana: general review throughout preliminary results

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Abstract. Since last decades, the physicians working in French Guiana, an Amazonian tropical area must consider a new clinical entity classically Amazonian toxoplasmosis (AT). This is a severe form of systemic acquired toxoplasmosis among immunocompetent adults with life-threatening pulmonary involvement and a greater potential for death. It involves emerging strains from the wild environment exhibiting high genetic diversity belonging to different haplogroups. Throughout the research studies we develop we are trying to improve our knowledge on the pathogenicity of these Amazonian *T. gondii* strains (ATG). We present some preliminary results on the epidemiology of toxoplasmosis in Human and animals, the murine virulence, immunological exploration and phylogenetic analysis.

Funding. We want to thank the "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01) and from a Fond Européen de Développement Régional grant (FEDER présage n°30820). They all contribute to financially support the different studies we carried on.

223. Transcriptomic analysis of human peripheral blood mononuclear cells (PBMC) stimulated ex-vivo with *Toxoplasma gondii*

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Abstract. *T. gondii* infects 25% of the world population. The majority of studies on this infection have been carried out in the mouse model being a limited model to infer about the human. The PBMCs have been used as a model but represent a great technical challenge, mainly in relation to differential gene expression studies. We designed an *ex vivo* model of stimulation with *T. gondii* RH-GFP using cells (PBMCs) from people with toxoplasmosis. After the stimulation, dual RNAseq was performed. Four independent trials were included. The first and second trials were taken for seronegative and seropositive individuals for *Toxoplasma* and the viability of the PBMCs was evaluated over time. In another trial, the PBMCs of individuals seropositive and seronegative for *Toxoplasma* were obtained to evaluate the different MOIs of infection (1: 1, 1: 3 and 1: 5), and a last test where the infection times of the infection model were evaluated. (1h, 6h, 24h). All infection data were analyzed by fluorescence microscopy. It was found that the viability of the *ex vivo* model of PBMCs is reduced by 30% from the fourth day, infection by *T. gondii* in PBMCs is dose-dependent and significant differences were found.

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224. Vaccine Approach with the aid of synthetic biology to control *Toxoplasma gondii*

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Abstract. Toxoplasmosis is zoonotic and parasitic disease which is widespread all over the world. The research was done for the expression of recombinant proteins of *T. gondii* namely SAG1, ROP18 and AMA1. With the help of bioinformatics, the sequences of the three genes; SAG1, ROP18 and AMA1 were retrieved from the Genbank. They were first aligned and consensus sequences were generated. The pET vector is selected for synthetic biology. The company was ordered to synthesize the sequences of SAG1, ROP18 and AMA1 in pET vector. The bacteria were transformed with the vectors and proteins were expressed BL21. SAG1 and ROP18 were expressed but AMA1 was unable to express. This was the approach to develop multi-protein sub-unit vaccine against *Toxoplasma gondii*. It is concluded that synthetic biology is one of the promising approach for the development of vaccine against *T. gondii*.

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225. Virulence of Amazonian atypical *Toxoplasma gondii* strains in a murine model

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Abstract. *Toxoplasma gondii*, an obligatory intracellular protozoan parasite of warm-blooded vertebrates is life-threatening usually during pregnancy and immunodepression status. Since recent decades, strains with particular phylogenetic profiles which differ from the clonal type circulating in North America and Europe (type I, II, III) have emerged especially in French Guiana. These strains, highly pathogenic have defined a new clinical entity called Amazonian Toxoplasmosis occurring mainly in immunocompetent persons. Using a murine model, we establish the type of virulence for these strains by comparison with three reference strains. Swiss mice were injected with different strains of *T. gondii* including the 3 above mentioned reference ones. We established a predictive virulence score in order to correlate the virulence of such strains with the severity of the disease in infected patients. All the indicators of virulence revealed that the Amazonian strains presented a comparable virulence profile to the referral highly virulent type I. The findings challenged upon the differences in virulence between human and animal strains, but also between anthropized and wild strains. Further studies must consolidate the relevance

of the results. Besides having a clinically relevant animal model of Amazonian toxoplasmosis, this model could also provide a solid experimental basis for future studies that will investigate the underlying mechanisms of Amazonian toxoplasmosis disease.

Funding. This work has benefited from an "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LA-BX-25-01) and from a Fond Européen de Développement Régional grant (FEDER présage n°30820).

226. Defining Host-Pathogen Interactions Employing an Artificial Intelligence Workflow

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Abstract. For image-based infection biology, accurate unbiased quantification of host-pathogen interactions is essential, yet often performed manually or using limited enumeration employing simple image analysis algorithms based on image segmentation. Host protein recruitment to pathogens is often refractory to accurate automated assessment due to its heterogeneous nature. An intuitive intelligent image analysis program to assess host protein recruitment within general cellular pathogen defense is lacking. We present HRMAN (Host Response to Microbe Analysis), an open-source image analysis platform based on machine learning algorithms and deep learning. We show that HRMAN has the capability to learn phenotypes from the data, without relying on researcher-based assumptions. Using *Toxoplasma gondii* we demonstrate HRMAN's capacity to recognize, classify and quantify pathogen killing, replication and cellular defense responses.

Funding. This work was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001076), the UK Medical Research Council (FC001076), and the Wellcome Trust (FC001076). EMF was supported by a Wellcome Trust Career Development Fellowship (091664/B/10/Z). DF was supported by a Boehringer Ingelheim Fonds PhD fellowship. AY, JM were supported by core funding to the MRC Laboratory for Molecular Cell Biology at University College London (J.M.), the European Research Council (649101-UbiProPox), the UK Medical Research Council (MC_UU12018/7).

227. Driving Forward: Linking Calcium and Parasite Motility

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Abstract. Calcium signaling is a universal signaling molecule that plays important roles in the regulation of a large number of cellular functions. Effector proteins have evolutionarily evolved to delicately balance either the rapid binding or release of calcium; and as such, oscillations in basal cytosolic calcium (Ca²⁺) induce a cascade of downstream processes. Ca²⁺ oscillations derive from extracellular Ca²⁺ influx and/or release from intracellular stores or some combination of both mechanisms working coherently. Within *T. gondii* Ca²⁺ signaling regulates the biological pathways that induce progression throughout the lytic cycle which is linked to the parasite's pathogenesis. As an obligate intracellular parasite, *T. gondii* must "sense and adapt" to changes in its surrounding ionic composition in order to effectively progress throughout the lytic cycle. Using parasites expressing genetically encoded Ca²⁺ indicators, we describe the unknown role that the extracellular environment plays in

gulating the Ca²⁺ oscillations that govern motility, an essential component of the lytic cycle; and in particular, we focus our attention on extracellular Ca²⁺ influx. We provide results highlighting recent advancements of our custom-made computer algorithm, used to simultaneously track and quantify parasite motility, as a proxy to study how pharmacologically blocking extracellular Ca²⁺ influx adversely impacts motility. Additionally, we provide data utilizing electrophysiology and fluorescent imaging to characterize the role of extracellular K⁺ (with and without extracellular Ca²⁺) plays in egress and motility.

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228. How does ROP23 Contribute to *Toxoplasma* pathogenesis?

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Abstract. *Toxoplasma gondii* is an intracellular Apicomplexan responsible for the zoonotic disease toxoplasmosis. *T. gondii* utilizes the contents of specialized secretory organelles at its apical end to exert its virulence. Specifically, *T. gondii* injects proteins (ROPs and RONs) from the rhoptry organelles directly into its host during invasion. While many rhoptry proteins have been implicated in the parasite's pathogenic processes, only a handful have been fully characterized to date. Our project focuses on the putative rhoptry protein, ROP23. Though expressed in all phases of infection, rop23 transcripts are significantly increased during the chronic stage of toxoplasmosis. Given the functions of known rhoptry proteins to *T. gondii* virulence, we postulate that ROP23 is directly secreted into the host cell from the rhoptries where it modulates host processes. To address this hypothesis, we generated an insertional mutant parasite line at the rop23 locus (*Drop23*), which had no significant on growth in vitro. However, preliminary data in a murine model of toxoplasmosis indicate that ablation of rop23 renders the parasite avirulent in CBA/J mice. To determine how ROP23 contribute to virulence, we generated an HA-tagged version of the protein for localization studies. Immunofluorescence assays, however, showed no indication of ROP23-HA in tachyzoites. We are currently assessing the expression of ROP23-HA in bradyzoites.

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229. A base-exchange type phosphatidylserine synthase is essential for the lytic cycle of *Toxoplasma gondii*

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Abstract. Phosphatidylserine (PtdSer) is a universal lipid, which is involved in various functions such as, apoptosis, membrane potential, protein sorting and secretion in mammalian cells. PtdSer also serves a precursor for other phospholipids. Many of its subcellular roles depend on the acidic nature and negative charge of this lipid, which allow it to interact with calcium and cationic domains of several proteins, and to help maintain the membrane potential across organelle membranes. Herein, we have identified the parasite enzyme underlying PtdSer synthesis in tachyzoites of *T. gondii*. The protein localizes in the membranes of the endoplasmic reticulum. Our inability to delete the PSS gene suggests its essential nature during the lytic cycle, which is also consistent with the phenotypic score (-4.8) in ToxoDB. Conditional regulation of PSS by tetracycline or Shield1 significantly reduced the synthesis and amount of PtdSer in tachyzoites, confirming its role in PtdSer biogenesis. Surprisingly, however, both mutants survived almost complete depletion of PSS concurrent with a reduction in PtdSer, likely due to compensation by other lipids including phosphatidylthreonine and posttranslational control of PSS activity. In further work, PSS gene was tagged with an auxin-inducible degradation (AID) domain, which yet again did not result in a severe phenotype. We are now implementing a Cre-LoxP-based gene excision method to demonstrate the essentiality of PSS gene. Our work also involves making double mutants of PSS and PtdThr synthase to resolve the functional relationship between the two related lipids.

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230. A Golgi-resident phosphatidylinositol synthase utilizing exogenous myo-inositol and endogenous CDP-diacylglycerol is essential for the lytic cycle of *Toxoplasma gondii*

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Abstract. Phosphatidylinositol (PtdIns) serves as an integral structural component of the eukaryotic membranes, as well as a precursor for the signaling mediators (phosphoinositides) and glycosylphosphatidylinositol anchor. PtdIns and its derivatives have been implicated in regulating the reproduction of apicomplexan parasites; the mechanism and physiological importance of PtdIns biogenesis remain to be understood however. Here, we demonstrate the presence of a novel PtdIns synthase (PIS) in a prevalent parasitic protist *Toxoplasma gondii*. TgPIS, encoding a functional enzyme with a catalytically vital CDP-alcohol phosphotransferase motif, resides exclusively in the Golgi body. Unable to synthesize myo-inositol de novo, the parasite imports it from milieu, and co-utilizes with CDP-diacylglycerol to produce PtdIns in a temporal and concentration-dependent manner. By contrast, *T. gondii* is unable to salvage sufficient PtdIns from its environment, which could compensate for the genetic deletion of TgPIS, suggesting a critical role of de novo lipid synthesis. An auxin-inducible conditional repression of TgPIS abrogates the lytic cycle of the parasite in mammalian cells due to defects in the replication, motility and egress. Lipidomic profiling of the PIS mutant demonstrates selective reduction of certain PtdIns species, while revealing a compensatory diversion of CDP-DAG to corresponding PtdGro and PtdSer species. Taken together, the results demonstrate a strict dependence of the parasite on autonomous PtdIns synthesis as opposed to scavenging, which can be exploited to develop anti-parasitic drugs.

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POSTER SESSION II

231. A role for *Toxoplasma gondii* chloroquine resistance transporter in bradyzoite digestive vacuole maintenance and viability

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Abstract. *Toxoplasma gondii* (*T. gondii*) is a ubiquitous pathogen that can cause encephalitis, congenital defects, and ocular disease, and has been implicated as a risk factor for mental illness in humans. The parasite persists in the brain as slow growing bradyzoites cysts. No treatments exist to eliminate this form of parasite. Although proteolytic degradation within the parasite's lysosomal-like vacuolar compartment (VAC) is critical for bradyzoite viability, whether other aspects of the VAC are important for parasite persistence remains unknown. An ortholog of *Plasmodium falciparum* CRT has previously been identified in *T. gondii* (TgCRT). To interrogate the function of TgCRT in chronic stage bradyzoites and its role in persistence, we knocked out TgCRT in a cystogenic strain and assessed VAC digestion of autophagosomes, host-derived proteins, VAC size, and viability of *in vitro* and *in vivo* bradyzoites. We found that whereas bradyzoites deficient in TgCRT exhibit near normal digestion of autophagosomes, they display a markedly distended VAC and their viability is compromised both *in vitro* and *in vivo*. Interestingly, impairing VAC proteolysis

in TgCRT deficient bradyzoites restored VAC size, consistent with a role for TgCRT in mobilizing products of digestion from the VAC. In conjunction with earlier studies, our current findings suggest a functional link between TgCRT and VAC proteolysis. This work provides further evidence of a crucial role for the VAC in bradyzoite persistence and a new potential VAC target to abate chronic *Toxoplasma* infection.

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232. An *in vitro* system to study heteroresistance and metabolic host interaction on mature tissue cysts of *Toxoplasma gondii*

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Abstract. Persisting stages of *T. gondii* are a key source of transmission and acute toxoplasmosis but cannot be targeted with currently available treatments. Because access to mature cysts is limited to *in vivo* models the mechanistic basis of this resistance is hard to study and identification of compounds that target tissue cysts efficiently remains challenging. To address both problems we optimized the generation of mature *T. gondii* cysts *in vitro*. To this end we infected terminally differentiated human myotubes as natural host cells with a wide range of parasite strains. Indeed, these cells support long-term culture of cysts, including type I RH parasites, without interfering growth of tachyzoites. Confirming their phenotypic similarity to *in vivo* cysts, our cysts lack expression of the tachyzoite antigen SAG1, develop pepsin resistance after three weeks and survive one week-long treatments with high doses of Pyrimethamine, Sulfadiazine, the quinolone HDQ and bumped-kinase inhibitors against CDPK1. Our EM-based ultrastructural analyses indicate broad distribution of polysaccharide stores, parasite packaging densities and proliferation within the same cyst, suggesting a heteroresistance mechanism against these compounds. Interestingly, host cell mitochondria associate with the vacuoles of type 2 parasites in these cells. This contrasts observations in fibroblasts and we are currently investigating implications on lipid uptake using mass spectrometry-based metabolomics. Summarized, we identified a human host cell line that can be used to raise *T. gondii* tissue cysts that are functionally similar to *in vivo* cysts. Our method will facilitate future studies on bradyzoite biology and enable the identification of bradyzoite compounds.

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233. Exploring possible interactions of dihydroorotate dehydrogenase with mitochondrial proteins in *Toxoplasma gondii*.

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Abstract. In higher eukaryotes, dihydroorotate dehydrogenase (DHODH) is associated with the inner mitochondrial membrane and transfers electrons to ubiquinone in the respiratory chain. Recent work by Fang and coworkers (2013, 2016), shows DHODH depletion causes mitochondrial dysfunction, and demonstrates apparent physical interactions of DHODH with respiratory complexes II and III. In *Toxoplasma gondii*, attempts to delete TgDHODH have failed, however, replacement of the endogenous gene with a catalytically deficient TgDHODH induces uracil auxotrophy (Hortua Triana et al., 2016), suggesting that TgDHODH possesses a second essential role that is not directly

dependent on enzyme activity. The goal of the present study is to analyze if there are any interactions between TgDHODH and mitochondrial membrane proteins that might point to alternate roles of this enzyme in the parasite. We have visualized the activities of TgDHODH, and respiratory complexes I, II, III, and IV using specific substrates in tachyzoites and on native gels. We will use these assays together with Western blots to determine whether TgDHODH co-migrates with membrane complexes from parasite mitochondria fractionated by Clear Native Polyacrylamide Gel Electrophoresis (PAGE), and 2D Blue Native PAGE. Additionally, we will use tagged TgDHODH to perform pull-down assays with parasite mitochondrial extracts.

Funding. Universidad de los Andes

234. Identification and characterization of novel host mitochondria-recruitment factor of *Toxoplasma gondii*

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Abstract. *Toxoplasma gondii* recruits some host organelles such as mitochondria and endoplasmic reticulum (ER) on its parasitophorous vacuole (PVM). Mitochondrial association factor 1 (MAF1) has been recently found as a mitochondrial recruitment factor (Pernas 2014). However mutant parasites in which MAF1 gene is knocked out can recruit some mitochondria around the PVM. Further, we reported that too much injection of rhoptry content induces higher rate of host mitochondrial recruitment (Tahara 2016). Considering them, we reasoned there were other recruitment factors in rhoptry proteins. To find new factors, we performed a quantitative mass spectrometry analysis of rhoptry proteins on the host mitochondria in the host cell infected with the parasite, because recruitment factors should bind to the host mitochondria. As a result, we could detect one rhoptry protein, ROP39, in the mitochondrial fraction. We deleted ROP39 gene using CRISPR/Cas9 system in *T. gondii* RH strain and observed its ability of host mitochondrial recruitment. We found the decrease of recruitment ability on ROP39 gene knock-out parasite in confocal and electron microscopy. Knock-out parasite increased the proliferation *in vitro* and decreased the virulence to mouse. ROP39 transfected into mammalian cell directly localized to host mitochondria. From these results, we concluded that ROP39 is one of the mitochondrial association factors.

Funding. This work was supported in part by Grants-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan

235. *In silico* and Experimental Study of SRS12B Protein of *Toxoplasma gondii*

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Abstract. The SRS proteins of *Toxoplasma gondii* are a very important proteins because they mediate the attachment of the parasite to the host cell and stimulates host immunity. We identified an adhesin *in silico* using Api-PredictorAdUniQE 2.0 and refining the search by a strategy in Toxo DataBase. There were not structural or functional role for the protein the candidates. The SRS12B protein of *T. gondii* was the candidate that we were able to study structurally *in silico*, to clone, to express *in vitro*, to purify and carry out the first experimental assay related to its possible function as adhesin protein. Until now, we obtained the following preliminary results: the structural analysis gave us structural information where SRS12B consists of two SAG-typical domains at both ends, presenting characteristic of members of the family. SRS12B is a homodimeric protein, the interface of the D1 domains show a defined groove for interaction with the ligand. The docking with heparin had a coupling free energy of -12.5 Kcal/mol, indicating a strong interaction of the ligand. In experimental assays, the plasmid pEXP5-CT / TOPO was successfully cloned with the coding region for SRS12B in *E. coli* One Shot TOP10 and later in *E. coli* BL21 DE3. The purified protein was used for co-precipitation assays that shows an interaction of this protein with cell membrane proteins of the Muller cell line that may correspond to cell membrane integrins. Finally, it is noteworthy that this research is oriented to the search for promising molecules as possible targets that contribute to an effective treatment against toxoplasmosis.

Funding. GEPAMOL, Center for Biomedical Research (CIBM), University of Quindío.

236. *In silico* Identification and Expression Profiling of the Protein Disulfide Isomerase Gene Family in *Toxoplasma gondii*

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Abstract. With one-third of the world's population infected by *Toxoplasma gondii*, opportunistic infections caused by this parasite are a frequent cause of clinical complications in immunocompromised individuals. Current treatments are focused on acute infections and are not effective against all stages of the parasite life cycle. To investigate new pharmacological targets, we began with a comparison of multistage gene expression over the life cycle of the parasite using *in silico* approaches. Comparison with other coccidian species revealed that the protein disulfide isomerase (PDI) family might be an important target for these parasites. We identified 20 proteins from *T. gondii* ME49, 20 with synteny in *N. caninum*, *S. neurona*, *H. hammondi* and 3 in *E. tenella*. These proteins contain at least one thioredoxin domain, as is characteristic of the PDI family, with variable numbers of thioredoxin domains in different architectures and combinations. Also, we identified proteins in the *T. gondii* life cycle that are expressed only during specific stages of the life cycle. We found that the protein TGME49_211680 has multistage expression and is the most expressed in the archetypal strains in both human cells and mice in chronic and acute stages. Invasion assays in human foreskin fibroblast cells with bacitracin, a known inhibitor for PDI proteins, showed reduced invasion percentages of tachyzoites. These results suggest that the PDI family has a role in tachyzoite invasion and could be a target for new therapeutic interventions. Furthermore, due to the high sequence conservation between coccidians, PDIs could also be targeted for different coccidiosis

Funding. Universidad del Quindío, EUPTH DB, University of Georgia.

237. *In silico* identification and *in vitro* evaluation of an inhibitory peptide for ROP5 protein of *Toxoplasma gondii* RH derived from the murine Irgb2-b1-CIM protein

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Abstract. *T. gondii* is an obligate intracellular parasite, responsible for toxoplasmosis, which is estimated to have infected a third of the human population, as well as being able to cause life-threatening disease in immunodeficient individuals or in individuals infected congenitally. This parasite has evolved several strategies to evade immune responses in many hosts. For example, ROP5 in conjunction with ROP18 act to block innate immune mechanisms activated by IFN- γ in murine hosts such as IRG proteins. Indeed, ROP5 binds to IRG6 in order to alter its structure and to expose residues that are phosphorylated by ROP18 leading to its inhibition. Recently, it has been demonstrated that CIM mouse can resist the infection with type I virulent RH parasites, and this phenotype was linked to the highly polymorphic tandem protein Irgb2-b1. Here, we identified a peptide (20 aa) derived from the helix 4 of Irgb2-b1-CIM through Time-Frequency Analysis. This peptide displayed a great uptake in iMEF cells, determined by immunofluorescence, and can interact with purified ROP5 according to a sandwich ELISA test. It was not cytotoxic at the concentrations evaluated (5 μ M - 150 μ M) by an MTT assay. It also showed the potential to decrease the parasite replication of RH-YFP parasites in IFN- γ stimulated iMEF cells, measured by fluorescence intensity reading, using a concentration of 50 μ M. This work describes a 20-mer peptide derived from the murine Irgb2-b1-CIM protein with promising inhibitory replication activity and non-toxicity.

Funding. Funding awarded by COLCIENCIAS Colombia through grant 1113-744-55483. Tobias Steinfeldt & Team at the Institute of Virology, Universitätsklinikum Freiburg.

238. *In silico* identification of dual inhibitors of TgCDPK1/TgCDPK3 as anti-*Toxoplasma* pharmacological alternatives.

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Abstract. *Toxoplasma gondii* is one of the most successful parasites worldwide due to its ability to infect a broad range of warm-blooded animals. In *T. gondii* there is a family of Calcium-dependent protein kinases which has been described as important for the life cycle of parasite and it lacks of orthologues in metazoans; this argument is what makes them targets of interest for drug design in protozoans. Up to date, 12 CDPKs are describe in *T. gondii*, within them, the TgCDPK1, TgCDPK3 and TgCDPK7, these have been studied due to their key roles in vital processes of the parasite. In the present, work we realized a computational analysis aimed to propose drug-like compounds as dual inhibitors of TgCDPK1 and TgCDPK3, having in consideration the protein sequence similarity of up to 51% between them. We downloaded crystallographic structures of both proteins (PBD: 3SX9 and 3HZT respectively) in order to perform a virtual screening from 642.000 compounds. Toxicity predictors (Datawarrior software and ProToxII web-server) for carcinogenic, mutagenic, tumorigenic, cytotoxic, neurocitotoxic and hepatotoxic effects were evaluated to reduce the number of compounds. Then, molecular docking studies were carried out to observe the interactions of the candidate compounds using Autodock Tools and Autodock Vina software to prepare the ligands and proteins. Finally, our results suggest that after our filters 31 compounds may be candidates for use in more rigorous computational studies and subsequent *in vitro* tests.

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239. *In vitro* evaluation of new 2-hydrazono-thiazolidinones against *T. gondii* proliferation

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Abstract. The generic therapy for toxoplasmosis has been a combination of antifolates. For acute toxoplasmosis infections, antifolates such as pyrimethamine (PYR) with sulfadiazine (SUL). (4,5) and, considering this mechanism of action, PYR-SUL treatment causes severe anemia and consequently, is usually administered simultaneously with folic acid (leucovorin) for preventing this side effect since humans (unlike *T. gondii*), can use exogenous folic acid for their cells (6). Even though this treatment is characterized by severe side effects: hypersensitivity, hematological toxicity, teratogenicity, allergic reactions; also the development of resistance has been identified, furthermore, susceptibility to PYR varies among the different *T. gondii* strains. On the other hand, thiazolidin-4-one derivatives have been studied since they have a broad spectrum in their biological activity, which makes them possible compounds with a promising pharmacological potential (7). Structural diversity is the key to discover new bioactive chemical entities with biological relevance. Modifications in the 4-thiazolidinone derivatives not only possess an inhibitory activity against the parasite but also a high selectivity-level with high therapeutic indexes, For all the above, we applied structure variations in 2-hydrazono-thiazolidinones core. These molecules were tested *in vitro* for the ability to inhibit tachyzoite growth over a period of 48h days with a modified fluorometric assay. The parasites that were glowing green were considered viable. The percentage of viable cells was calculated using the following formula: (fluorescence of the sample with treatment URF * 100) / fluorescence of the control without treatment URF = (sample Fluoresce*100)/BLKnotto) been URF unit relatives of fluorescence. by fluorescence

Funding. COLCIENCIAS

240. *In vivo* control of *Toxoplasma gondii* by zebrafish macrophages

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Abstract. *Toxoplasma gondii* is an obligate intracellular parasite capable of invading any nucleated cell, and infects 30% of the human population. Murine models have driven the understanding of immune mechanisms underlying *Toxoplasma* infection, but are limited for studying real-time parasite-leukocyte

interactions *in vivo*. Moreover, discrepancies exist between the innate cellular responses observed in mouse versus human cells *in vitro*. Here, we establish a zebrafish infection model to study *in vivo* the innate immune response to *Toxoplasma* tachyzoite infection. We show that intracellular *Toxoplasma* can replicate and survive in the zebrafish hindbrain up to 48 hours post-infection. Using 3D correlative light and electron microscopy (CLEM) we show intramacrophage parasite replication and recruitment of host mitochondria to the parasitophorous vacuole, a hallmark of type I *Toxoplasma* infection observed in mice. Strikingly, a comparison of three different *Toxoplasma* strain types *in vivo* reveals significant differences in the establishment of infection and macrophage response. Using fluorescence microscopy, we characterise macrophage-parasite interactions and show that macrophage depletion results in significantly increased parasite burden. Our results so far strongly suggest that macrophages have an important role in zebrafish host defence against *Toxoplasma*. Having established a zebrafish model of *Toxoplasma* infection, we now have an *in vivo* infection platform for CRISPR targeting and high throughput drug screens that, together with real-time microscopy, can be used to identify novel determinants underlying *Toxoplasma* infection control.

Funding. This work was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001999 to LC, FC001076 to EMF), the UK Medical Research Council (FC001999 to LC, FC001076 to EMF), and the Wellcome Trust (FC001999 to LC, FC001076 to EMF). EMF was supported by a Wellcome Trust Career Development Fellowship (091664/B/10/Z). Research in the Mostowy laboratory is supported by a Wellcome Trust Senior Research Fellowship (206444/Z/17/Z), Wellcome Trust Research Career Development Fellowship (WT097411MA), and the Lister Institute of Preventive Medicine.

241. Mechanisms of Apicoplast Division in *Toxoplasma gondii*

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Abstract. During *Toxoplasma gondii* cell division, the single copy organelles such as the Golgi, Mitochondria and Apicoplast must divide, so one copy can be inherited by each daughter. Given the importance of these organelles to parasite survival, the process of organelle division and inheritance must occur with high accuracy. Apicoplast division is a multi-step process: First, the apicoplast elongates and associates with the duplicated centrosomes. As daughter parasites grow, the apicoplast forms a U-shaped with each end anchored at the apical end of each daughter. Finally, apicoplast fission at the base of the apicoplast allows one apicoplast to be inherited by each daughter. It has recently been demonstrated that loss of an unconventional myosin, Tg-MyoF and actin result in apicoplast inheritance defects. Our goal is to elucidate the mechanism by which these proteins facilitate apicoplast inheritance. We hypothesized that TgMyoF and actin could provide the force for apicoplast elongation, however our preliminary data indicate that actin depolymerization with cytochalasin D does not affect apicoplast elongation. Currently, we are using immunofluorescence assays and live cell imaging to identify the step in the apicoplast division cycle that is dependent on TgMyoF and actin.

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242. Modulation of arginase 1 in RAW 264.7 and peritoneal macrophages after *Toxoplasma gondii* infection

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Abstract. *Toxoplasma gondii* is an obligate intracellular parasite with widespread distribution being able to infect distinct nucleated cell. It has been proposed that *T. gondii* induces arginase 1 (ARG1) expression in host cells as an immune evasion strategy, but the role of this enzyme for *T. gondii* development in non-activate or alternatively activated mouse macrophage cell lines is not known. Here, we evaluated the activity of ARG1 in non-activate and alternatively activated RAW 264.7 and Swiss peritoneal mice macrophages after infection with *T. gondii*. The role of ARG1 in the development of *T. gondii* in these cells was also determined. ARG1 activity was higher in alternative activated macrophages and in peritoneal macrophages when compared to RAW 264.7. After infection, ARG1 activity enhanced in non-activate RAW 264.7 and peritoneal macrophages; but did not alter in alternatively activated macrophages. L-arginine supplementation of culture medium favored *T. gondii* replication in

RAW 264.7 macrophages and treatment of these cells with Nor-NOHA (ARG1 inhibitor) reduced the infection rate of *T. gondii* in a dose dependent manner especially in alternative activated cells. Our findings indicate that the enhancement of ARG1 activity may be a general mechanism induced by *T. gondii* to replicate inside macrophages.

Funding. UENF, FAPERJ, CNPq, CAPES.

243. Optogenetic illumination of cAMP signaling in *Toxoplasma gondii*

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Abstract. Infection and pathogenesis of *Toxoplasma gondii* depend on coordinated signaling by cAMP and cGMP. To synthesize cAMP, the parasite encodes four adenylate cyclases (TgAC α 1, TgAC α 2, TgAC α 3 and TgAC β). Individual deletions of TgAC α 1-3 genes in tachyzoites does not show an apparent defect in parasite growth, while knockout of TgAC β causes about 70% reduction in plaque size. We were able to compensate the absence of TgAC β by expressing a photoactivated adenylate cyclase from a lithotropic bacterium *Beggiatoa* (bPAC-S27A-HA) in the cytosol of tachyzoites. More importantly, the light-sensitive strain allowed specific, fast, spatiotemporal and reversible induction of cytosolic cAMP by blue light-emitting diodes (455 nm) in intracellular and extracellular parasites, which is just not feasible by existing methods. A brief illumination of extracellular tachyzoites caused a maximal 17-fold induction in cAMP that could be fine-tuned by light intensity, duration and wavelength, as needed. Phenotyping of the optogenetic strain specified a role of cAMP during invasion, which was reduced by 10x upon transient illumination. Contrariwise, we observed an enhanced egress (2-fold) by light. Phosphoproteomics of optogenetic strain identified a total of 1061 phosphopeptides (consensus of 3 assays, p

Funding. We thank to *Toxoplasma* community for sharing reagents. This work was funded by a grant (GU1100/7-1) to NG, awarded by the German Research Foundation.

244. Studies of ApiAP2 factors reveal important features of the timing and assembly of tissue cysts

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Abstract. Our investigations demonstrate ApiAP2 transcription factors serve to promote and repress bradyzoite gene expression. The ApiAP2 factor, AP2IV-4, is a dynamic cell cycle regulated factor of the tachyzoite that represses the expression of tissue cyst wall components BPK1, MCP4 and CST1. Another transcriptional repressor, AP2IX-9, also acts to prevent the bradyzoite development through inhibition of metabolic gene expression such as LDH2 and ENO1. Disrupting AP2IV-4 de-represses the expression of cyst wall proteins in tachyzoites, which profoundly changes the innate immune response in mice. Mouse inflammatory macrophages are specifically recruited in response to infection with these altered tachyzoites, which we believe are responsible for the quick clearance of the parasite. Importantly, the high levels of cyst wall proteins such as CST1 in tachyzoites that lack AP2IV-4, does not lead to assembly of a cyst wall. CST1 is exported into the parasitophorous vacuole and forms a cyst wall only after alkaline stress indicating there are factors induced during bradyzoite development that are required for tissue cyst wall assembly. Disruption of both AP2IV-4 and AP2IX-9 further increases cyst wall protein depression through a mechanism that is not understood. The single deletion of AP2IX-9 also showed delayed cyst wall assembly in alkaline media suggesting that AP2IX-9 might work to promote posttranslational modifications of CST 1 such as glycosylation or transferring CST1 to the PVM membrane layer. Altogether our results indicate that bradyzoite tissue cyst wall formation requires the inactivation of tachyzoite transcription factors and the induction of assembly factors.

Funding. This work was supported by grants from NIH NIAID.

245. The Inositol polyphosphate pathway of *Toxoplasma gondii*

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Abstract. Inositol polyphosphates are a diverse class of intracellular messengers present in eukaryotic cells. Myo-inositol is the basic building block with six possible positions for phosphorylation. The fully phosphorylated form is known as phytic acid. Further addition of diphosphates to the ring results in the production of inositol pyrophosphates. These water-soluble and high-energy small molecules are involved in critical cellular functions such as vesicle trafficking, phosphate homeostasis, ribosome synthesis and stress response. Inositol pyrophosphates play roles in signaling and regulation of gene expression. Inositol polyphosphates pathway has not been studied in *T. gondii*, however, genes that encode three of the inositol phosphate kinases of the pathway are present in its genome. We cloned, expressed and purified the recombinant protein of two of these enzymes. Inositol polyphosphate multikinase (TgIPMK), enzyme with dual activity that catalyzes the conversion of inositol trisphosphate (IP3) into inositol-tetrakisphosphate (IP4) and inositol-pentakisphosphate (IP5); and the hexakisphosphate kinase (TgIP6K), that uses inositol-hexakisphosphate (IP6) to produce inositol pyrophosphates. We showed that both enzymes have kinase activity. TgIPMK was able to use IP3 as substrate to produce IP5 and, TgIP6K can use IP5 to produce diphosphoinositol-tetrakisphosphate (PP-IP4) and IP6 to produce 5-diphospho-inositol-pentakisphosphate (5-IP7). We investigated subcellular localization of TgIPMK and TgIP6K by endogenous tagging and found cytosolic localization in extracellular tachyzoites. Furthermore, using the tetracyclin-regulated transactivator expressing strain (Tat1 Δ ku80) we developed conditional knockouts of TgIPMK (Δ TgIPMK) and TgIP6K (Δ TgIP6K) and showed that these enzymes are essential for parasite growth. This is the first evidence that protein pyrophosphorylation occurs in *T. gondii*.

Funding. Center for Tropical and Emerging Global Diseases, University of Georgia National Institutes of Health (NIH) Office of Research, UGA

246. Unsupervised *Toxoplasma gondii* Recognition by Fuzzy Cycle Generative Adversarial Network

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Abstract. *Toxoplasma gondii*, one of the world's most common parasites, can infect all types of warm-blooded animals, including one-third of the world's human population. Most of current routine methods of diagnosis are costly, time-consuming, and labor-intensive. The parasite can be directly observed under the microscope in tissue or spinal fluid samples, and this form of testing is cheap, but used less frequently because of the difficulty in operation, and needs well trained professionals. Nevertheless, observation of parasites in patient or animal specimens under the microscope is still performed by a large number of laboratories. The identification of *Toxoplasma* parasite with cost-effectiveness solution is still a health challenge in developing countries. Here, we propose an Artificial Intelligence (AI)-powered microscopic image analysis method for *Toxoplasma gondii*. The method uses Fuzzy Cycle Generative Adversarial Network (FCGAN) with transfer learning, by applying knowledge gained by the parasitologists that *Toxoplasma gondii* is in banana or crescent shaped form. Our approach aims to build connection between micro and macro associated objects by embedding fuzzy c-Means cluster algorithm into Cycle GAN. We show the high accuracy and effectiveness of our approach in the newly collected 28,130 unlabeled microscopic images from *Toxoplasma* infection samples.

Funding. School of Science, Harbin Institute of Technology, Shenzhen

247. Evaluation of antiparasitic activity from raw extracts of *Passiflora edulis*, *Selaginella geniculata*, *Cannabis sp* in *in vitro* culture of *Toxoplasma gondii*

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Abstract. Parasitic diseases continue to be one of the main problems in public health in our country. Chemicals synthesized in the laboratory can't be used because of their toxicity or effects are constantly increasing. This is how the use of medicinal plants as an alternative for the control of these diseases. The antiparasitics used for the infection produced by *Toxoplasma* are toxic to some degree to humans. Therefore, we have evaluated the antiparasitic activity from extracts of *Selaginella geniculata*, *Passiflora edulis* and *Cannabis* sp in *T. gondii* culture. We performed an *in vitro* evaluation of the effect on *T. gondii* culture invasion and growth, according to the protocols of the GEPAMOL group. After establishing the culture, assays with 10,000 cells in 96-well plates were performed and different concentrations of crude extracts were used (320 µg/ml, 100 µg/ml, 50 µg/ml and 1.7 µg/ml). The biological compounds underwent a cytotoxicity assay to determine cell viability using Resazurin as an indicator in a cell line (HFF). The effect of the extracts on the cells infected with the parasite is also evaluated. As a result of cytotoxicity only one of the concentrations (320 µg/ml) of *S. geniculata* was not optimal for subsequent trials. However, no significant effect has been found in the extracts on the growth or invasion of *T. gondii* in HFF cells.

Funding: Universidad del Quindío

248. Modulation of arginase 1 in RAW 264.7 and peritoneal macrophages after *Toxoplasma gondii* infection

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Abstract. *Toxoplasma gondii* is an obligate intracellular parasite with widespread distribution being able to infect distinct nucleated cell. It has been proposed that *T. gondii* induces arginase 1 (ARG1) expression in host cells as an immune evasion strategy, but the role of this enzyme for *T. gondii* development in non-activate or alternatively activated mouse macrophage cell lines is not known. Here, we evaluated the activity of ARG1 in non-activate and alternatively activated RAW 264.7 and Swiss peritoneal mice macrophages after infection with *T. gondii*. The role of ARG1 in the development of *T. gondii* in these cells was also determined. ARG1 activity was higher in alternative activated macrophages and in peritoneal macrophages when compared to RAW 264.7. After infection, ARG1 activity enhanced in non-activate RAW 264.7 and peritoneal macrophages; but did not alter in alternatively activated macrophages. L-arginine supplementation of culture medium favored *T. gondii* replication in RAW 264.7 macrophages and treatment of these cells with Nor-NOHA (ARG1 inhibitor) reduced the infection rate of *T. gondii* in a dose dependent manner especially in alternative activated cells. Our findings indicate that the enhancement of ARG1 activity may be a general mechanism induced by *T. gondii* to replicate inside macrophages.

Funding. UENF, FAPERJ, CNPq, CAPES

249. The first isolation and molecular characterization of *Toxoplasma gondii* from pigeons in Bogotá, Colombia

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The domestic pigeon *Columba livia* is considered as a symbol and attraction in various public spaces. However, its possible role as a reservoir and transmitter of zoonotic diseases can turn them into a risk factor for public health. The objective of this study was to describe the *Toxoplasma gondii* infection in pigeon samples from Bogotá, Colombia. For this, 10 stool samples were taken, which were extracted from the intestines of the pigeons, the product was made the Ritchie method and the pellet obtained was washed with 1X

PBS. To extract the DNA, 1ml of the obtained product was taken; *Toxoplasma* spp cell lysate was performed in combination of mechanical lysis and chemical lysis. Next, the DNA was purified, following the protocol of the Wizard Genomics Kit (Promega). For the detection of the parasite the B1 gene was amplified by nested-PCR. Which consisted of a denaturation for 1 min at 94 °C, an alignment of 30 seconds at 53 °C and extension 30 seconds at 72 °C. From the previous PCR product, Gen B1 was amplified with primers, 5'TGCATA-GGTTGCCAGTCACYG3' and 5'GGCGACCAATCTGCGAATACA3', to obtain a final amplification of 97 bp. Of the 10 samples processed, the 20% amplification was obtained. We conclude that we describe the first report in Colombia of *Toxoplasma gondii* for the species *Columbia livia*, which may represent a possible source of infection for humans, animals, food and the environment.

250. Meta-analysis of the association between toxoplasmosis and suicide attempt

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Abstract. Recent studies have linked toxoplasmosis to suicide. Here we subjected these studies to a meta-analysis. Published articles and abstracts were identified by searches of PUBMED and Web of Science. Published controlled studies that used serological methods for measuring *T. gondii* antibodies to assess in patients with suicide attempt and controls, were selected for analysis. Nine studies with primary data were identified and met the selection criteria. The combined odds ratio (OR) was 1.25 (95% confidence interval, 1.08 to 1.43; p<0.014). The results indicated that population with or without mental illness and suicide attempt have an increased prevalence of antibodies to *T. gondii*. This association occurred in population of different countries. Identification of the parasite in brain of people committing suicide, it is necessary to provide biological evidence about these epidemiological findings

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251. Hygiene-health factors associated with *Toxoplasma gondii* infection in children that assisted to school restaurants in Armenia, Quindío, Colombia.

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Abstract. We assessed the hygiene health factors associated in children that assisted to school restaurants in Armenia (Quindío, Colombia). We performed a questionnaire about hygiene habits at school and home in children that assisted to restaurants from public schools and responses were correlated with the prevalence of specific IgG anti-*Toxoplasma* antibodies. In 117 children who attended the restaurants, the most significant habit related to an increase in risk for *Toxoplasma* infection was the consumption of undercooked meat (OR 36; 95%CI: 4,5-288)). Education about food preparation in home it is necessary to reduce the risk of *Toxoplasma* infection in children.

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252. Clinical characteristics of human ocular toxoplasmosis are related to *Toxoplasma* serotype and host immune response.

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Abstract. The aim of this work was to evaluate a modified serotype method based on the use of polypeptides derived from the GRA6 protein and to determine the cytokine gene polymorphisms in human ocular toxoplasmosis, related to the clinical characteristics (number of lesions, severity of inflammation) to investigate whether a correlation can be established between the clinical manifestations, the immune response, the host gene polymorphism and the

parasite type. We performed an ELISA analysis of the *Toxoplasma* GRA6 serotype in 23 patients with ocular toxoplasmosis and 20 individuals chronically infected with *Toxoplasma* but without ocular involvement. In patients with ocular toxoplasmosis, we analyzed host gene polymorphisms related to immune response (IL-1 β ; IL-1 α ; IL-10; IFN- γ ; TNF- α , IL-12), IL-17R, TLR-9, and P2RX7. Additionally, eight patients were studied for the production of TNF- α , IL-1 β , IFN- γ and IL-10 by their peripheral leukocytes after *ex vivo* stimulation with soluble *Toxoplasma* antigens. Seropositivity for GRA6-I was associated with higher number of retinal lesions and higher levels of IL-1 β . Two polymorphisms were associated with specific clinical manifestations of OT: IL-10 -819 C/T with bilateral lesions, and IL-12 +169774 A/C, with synechia. Higher levels of IL-10 were found in patients with the allele G/G at the polymorphic region IL-10 -1082. People with a GRA6 I serotype and possessing the allele G/G at the polymorphic region TNF-857 suffered from an increased number of retinal lesions. We found a positive association between host cytokine genes polymorphisms and GRA6 serotypes correlated with specific clinical manifestations and immune response in ocular toxoplasmosis.

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253. *Toxoplasma gondii* sensing and regulated necrosis in IFN γ -primed host cells

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Abstract. Intracellular *T. gondii* resides inside the parasitophorous vacuole (PV), where it avoids detection and elimination. The innate immune response orchestrated by interferon gamma (IFN γ) is key to limiting parasite dissemination. Host cell priming with IFN γ activates cell-autonomous immunity (CAI). Mice rely on CAI effectors, the immunity-related GTPases (IRGs), whereas human cells utilize mainly guanylate-binding proteins (GBPs). In mouse cells, PV membrane (PVM) attack by IRGs (and GBPs) results in parasite death, swiftly followed by host cell regulated necrosis, a pro-inflammatory type of death. Pattern-recognition receptors (PRRs) detect pathogens directly, by recognizing pathogen-associated molecular patterns (PAMPs), or indirectly, by recognizing the result of cell death or tissue damage, so-called damage-associated molecular patterns (DAMPs). PRR engagement triggers immune responses that dictate the outcome of infection. Little was known about *Toxoplasma* detection by host PRRs, how parasite sensing drives cell death, or the necrosis mediated by avirulent *T. gondii* infection of IFN γ -induced mouse fibroblasts. Using genetic screens and cell biology tools, we showed that *Toxoplasma* sensing pathways are unconventional: parasite recognition does not require RLRs, TLRs or classical cytosolic DNA sensors, nor STING and MAVS. Moreover, host cells do not undergo canonical necroptosis. Crucially, we uncovered an unexpected role for DAMP receptors galectin-8 (and -9) in *Toxoplasma* sensing: galectins recognize glycans exposed to the cytosol upon PVM rupture and may further recruit selective autophagy adaptors. Galectins had been implicated only in vacuolar bacteria and adenovirus sensing. This unique model system will continue to afford us important insights into pathogen sensing/necrosis pathways.

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254. Host Genetic Susceptibility to Ocular Toxoplasmosis. A Systematic Review and Meta-Analysis of Cytokine Gene Polymorphisms Association Studies

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Abstract. Aims. The aim of this study was to perform a meta-analysis to assess and synthesize the available data from association studies of inflammatory cytokine polymorphisms on the risk to develop OT. Methods. An electronic search was conducted in PubMed, Scopus, Web of Science, and Virtual

Health Library databases through April 30 2018, without restrictions for year or language. All meta-analysis association tests were performed using the MetaGenyo software. The application allowed to obtain ORs with 95% CIs for polymorphisms of the IL-10 (rs1800896), IL-1 α (rs1800587), IL-1 β (rs1143634), TNF- α (rs1800629) and IFN- γ (rs2430561) genes related to OT susceptibility under a genetic allele model. All study heterogeneities were estimated using the Cochran's Q and the heterogeneity was considered significant at P

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255. Inflammatory gene expression profiling in peripheral blood from older people with chronic toxoplasmosis

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Abstract. Aims. This study aimed to explore the molecular pathogenesis of toxoplasmosis-related inflammation through next generation sequencing, to assess RNA expression profiles in peripheral blood from 5 patients with chronic toxoplasmosis and 5 healthy controls. Methods. 10 ml of peripheral blood were extracted. All serum samples were analyzed for anti-*Toxoplasma* IgG and IgM antibody titers by using electrochemiluminescence. Transcriptome sequencing was performed using the Ion AmpliSeq Transcriptome Gene Expression Kit. Pathway and process enrichment analysis of DEGs was performed using Metascape (metascape.org). Results. Differential gene expression analysis revealed 75 DEGs at a false discovery rate of 0.1, of which 46 were up-regulated and 29 were down-regulated considering values of p 0.5 and < -0.5. The top clusters with their representative enriched terms were GO:0060333 (interferon-gamma-mediated signaling pathway) Log10(P): -7.85 and GO:0001816 (cytokine production) Log10(P): -7.08. Pathway and process enrichment analysis has been applied to each MCODE component independently, and the first best-scoring term by p-value have been retained as the functional description of the corresponding component GO: 0035304 (regulation of protein dephosphorylation) Log10(P): -7.2. The top 10 most significantly upregulated genes were LILRB2, PRKCD, CYBB, GCA, CLEC4D, CD68, IRF1, MCEMP1, C3AR1 and SLC11A1. GeneMANIA (<http://genemania.org>). These gene-gene interactions are realized through co-expression (95.19%), co-localization (4.81%). Conclusions. Chronic *T. gondii* infection is associated with an inflammatory response. Possible associations of chronic *T. gondii* infection with a number of psychomotor and cognitive functions have been suggested to be altered in *T. gondii*-seropositive individuals.

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256. Revising the karyotype of *Toxoplasma gondii* and synteny with *Neospora caninum* using single molecule sequencing.

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Abstract. The *Toxoplasma* and *Neospora caninum* genomes still have multiple gaps due to repetitive and unclonable sequences. Here we report direct, single molecule sequencing and de novo assembly of the genomes for multiple *Toxoplasma* strains and *Neospora caninum* (Liverpool) using the MinION nanopore sequencer. We obtained an average of 650,000 reads per strain (~7.4 Gb of sequence) and more than 90% of the reads could be mapped to the reference genome. The resulting assembly improved *T. gondii* and *N. caninum* assembly contiguity (N50 of ~6.6Mb) and increased the overall assembled sequence by ~2Mb. For all de novo assemblies multiple complete chromosomes were fully assembled as evidenced by clear telomeric repeats on the end of each contig. Interestingly, for all of the *Toxoplasma gondii* strains that we sequenced (RH, CTG, II \times III F1 progeny clones CL13, S27, S21, and S26), the largest contig ranged in size between 11.9 and 12.1 Mb in size, which is larger than any previously reported *T. gondii* chromosome. This was due to a repeatable and consistent fusion of chromosomes VIIb and VIII and these data were supported by a previous report of a similar potential chromosomal fusion

in *T. gondii* based on HI-C data (Bunnik et al., PNAS 116:3183-3192). Finally, when we compared the *T. gondii* and *N. caninum* assemblies we found some chromosomes that had nearly 100% synteny while others lacked chromosomal synteny, suggesting that multiple large scale translocation events have occurred in *T. gondii* and *N. caninum* since their most recent common ancestry.

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257. QSAR and Molecular docking studies of 2-benzoyl-4-thiazolidinones derivatives as potential *Tg* ROP18 inhibitors of *Toxoplasma gondii*

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Abstract: The current regimen of therapy include combinations of antifolates and sulfates or antibiotics that are associated with high toxicity and low efficacy. The key participant in controlling virulence in both rodent and human hosts has been identified as TgROP18. Thiazolidinone derivatives present promising pharmacological potential for the treatment of *T. gondii* infections.

Novel thiazolidin-4-one analogs are reported recently as effective inhibitors with minor cell toxicity. This study specifically focuses on identifying potential inhibitors that towards targeting *Toxoplasma gondii*, to screen 4-thiazolidinones inhibitors to block the kinase activity of ROP18 and predictive quantitative structure-activity relationships (QSAR) of new 1,3-thiazolidin-4-one analogs against *Toxoplasma gondii*. We constructed three QSAR models, performed on 95 previously reported *T. gondii* inhibitors. The QSAR models were validated by the experimentally reported dataset ($R(2)p=0,80$). The models were employed to estimate the experimental IC₅₀ between 4-12 μ M. As a result, a series of 146 molecules were designed then docked with TgROP18. The structure of the thiazolidinone derivatives was obtained by energy minimization using the Gaussian 09 software; docking studies were carried out on 146 molecules and the most active molecule was analyzed in the receptor ligand-binding region with docking score between -8,9 and -13,5 kcal/mol. Identified 30 inhibitors with distinct scaffold types. The docking models and QSAR analysis demonstrated that these hits could engage in multiple hydrogen bonds and hydrophobic interactions with ROP18, and para-position halo substituents on the benzene and heterocyclic ring may enhance their affinity scoring.

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SUMMARY OF PROGRAM

XVth International *Toxoplasma* Congress (Toxo XV)

19 to 22 June 2019,
Decameron Panaca - Quindío

WEDNESDAY 19th JUNE 2019

1:00 – 3:00 p.m	Registration
5:00 – 5:30 p.m	Welcome words
5:30 – 7:15 p.m	Session I - Epidemiology and environmental studies Discussion Leaders: Patricia Conrad- David Fergusson
8:00 – 9:00 p.m	Keynote Speaker. "The new vaccines". Professor Manuel Elkin Patarroyo
9:00 p.m	Buffet dinner

THURSDAY 20th JUNE 2019

8:00 – 9:45 a.m	Session II - Immunology Discussion Leaders: Jonathan Howard- Eva Frickel
10:00 – 10:45 a.m	Coffee Break
10:00 – 12:15 p.m	Session III - Cell Cycle Discussion Leaders: Lilach Sheiner- Maria Francia
12:15 – 1:00 p.m	Lunch
1:00 – 2:00 p.m	Discussion groups
2:00 – 4:00 p.m	Session IV - Biochemistry I Discussion Leaders: Maryse Lebrun - Jeroen Saeij
4:00 – 4:30 p.m	Coffee Break
4:30 – 6:30 p.m	Session V - Genomics, transcriptomics and evolution Discussion Leaders: David Roos- Jessica Kissinger
6:30 – 7:30 p.m	Poster Session I
8:30 – 9:00 p.m	Dinner
9:00 p.m	Onwards Poster Session I

FRIDAY 21st JUNE 2019

8:00 – 10:00 a.m	Session VI - Host parasite interactions I Discussion Leaders: Martin Blume- Sebastien Besteiro
10:00 – 10:30 a.m	Coffee Break
10:30 – 12:00 p.m	Session VII - Immunology II Discussion Leaders: Nestor Cardona - Kirk Jensen
12:00 – 1:00 p.m	Lunch
2:00 – 4:00 p.m	Session VIII - Clinical toxoplasmosis. Discussion Leaders: Alejandra de la Torre - Alexander Pfaff
4:00 – 4:30 p.m	Coffee Break
4:30 – 6:00 p.m	Session IX - Trafficking pathways Discussion Leaders: Moritz Treeck- Vern Carruthers
6:30 – 7:30 p.m	Poster Session II
8:30 – 9:00 p.m	Dinner
9:00 p.m	Onwards Poster Session II

SATURDAY 22nd JUNE 2019

8:00 – 10:00 a.m	Session X - Host Parasite Interactions II Discussion Leaders: Aylan Arenas- Sebastian Lourido
10:00 – 10:30 a.m	Coffee Break
10:30 – 12:15 p.m	Session XI - Biochemistry II Discussion Leaders: Lena Pernas- John Boothroyd
12:15 p.m	Awards announcement – Farewell!

HOTEL MAP

DECAMERON
PANACA
Hotel, Centro de Convenciones, Parques y Spa
Región Cafetera de Colombia



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|----------------------------|---------------------------------------|------------------------------------|---|
| 1. LOOBY - BAR LA PESEBRRA | 7. SPA | 13. MÓDULO DE HABITACIONES No. 3 | 19. ENTRADA AL HOTEL DECAMERON PANACA |
| 2. RESTAURANTE LA ESTANCIA | 8. ESTABLO "LOS PONYS" | 14. MÓDULO DE HABITACIONES No. 4 | 20. ENTRADA A LAS VILLAS DECAMERON PANACA |
| 3. BAR KIOSKO | 9. CENTRO DE CONVENCIONES EL BOSQUE | 15. MÓDULO DE HABITACIONES No. 5 | 21. VILLAS DECAMERON PANACA |
| 4. PISCINAS | 10. CENTRO DE CONVENCIONES LA ALAMEDA | 16. PUENTE COLGANTE HACIA PANACA | 22. ESTACIONAMIENTO INTERNO |
| 5. SNACKS | 11. MÓDULO DE HABITACIONES No. 1 | 17. SALIDA Y LLEGADA DE CABALGATAS | PUNTOS DE ENCUENTRO |
| 6. RESTAURANTE LA HERRERÍA | 12. MÓDULO DE HABITACIONES No. 2 | 18. MULTIVACACIONES | |



INTERNATIONAL
TOXOPLASMA
CONGRESS
(Toxo XV)



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**XVIII CONGRESO LATINOAMERICANO
DE INFECTOLOGIA PEDIATRICA SLIPE 2019**

**XIV CONGRESO COLOMBIANO
DE ENFERMEDADES INFECCIOSAS**



21 al 24 de agosto de 2019
Centro Internacional de Convenciones Las Américas
Cartagena, Colombia



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