RT 01 - Survival Strategies in Trypanosomatids: From DNA Replication and Genomic Plasticity to Vector Transmission

RT.01 – 001 - **Deciphering the machineries that guide initiation and progression of nuclear DNA replication in *Trypanosoma brucei***

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All organisms must precisely duplicate their genome before cell division to ensure its faithful inheritance. DNA replication is a tightly regulated process in eukaryotes that starts by interaction of the origin recognition complex (ORC) at discrete sites in the genome, termed origins. Once bound to origins, ORC directs the sequential recruitment and activation of the DNA replication machinery, starting with the replicative MCM helicase. Only in some yeast has the mechanism of ORC-origin interaction been defined. DNA replication programming in trypanosomatids, including Trypanosoma brucei, faces several challenges, including the pervasive use of multigenic transcription and pronounced genome compartmentalisation and instability. To begin to understand how T. brucei tackles these challenges, we have used proximity labelling to provide spatial and pseudo-temporal analysis of DNA replication initiation and progression, providing several insights. A complete ORC, comprising six predicted orthologous subunits, has escaped detection in all kinetoplastids, but BioID of two putative ORC subunits revealed a highly diverged five subunit ORC, which Alphafold predicts to form a structurally conserved complex. In addition, BioID of an S-phase restricted ORC-like factor, ORC1B, suggested closer association with MCM helicase subunits than ORC, but is nonetheless predicted to complete ORC encirclement of DNA, suggesting a means for activation of DNA replication initiation. Across all three BioID datasets, we identified 39 shared proximal proteins, 12 of which are transcription-associated factors, consistent with previous work that indicated DNA replication initiates at transcription start and stop sites in the T. brucei genome core. Amongst these factors, we have demonstrated direct interaction between ORC4 and four of the transcription-related proteins, providing the first indication in any non-yeast eukaryote of how DNA replication origins are designated. Finally, we have used BioID to explore the protein environment of the moving replisome, providing validation of many so-far uncharacterised replication factors as well as recovery of potential activities needed to manage replication ‘stress’, such as abundant DNA repair activities. **Keywords:** trypanosome; DNA replication; proximity labelling.

RT.01 – 002 - **The Cost of Flexibility: Mapping Replication Stress Hotspots and their Impact on *Leishmania* Genomic Maintenance**

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Faithful genome duplication requires the coordinated progression of replication forks across every chromosome. However, forks inevitably encounter obstacles such as DNA lesions, protein–DNA complexes, repetitive DNA, active transcription and stable DNA secondary structures. These impediments generate replication stress (RS), now recognized as an intrinsic consequence of genome duplication rather than merely a response to exogenous damage. In Leishmania, the unusual organization of the genome into constitutively transcribed polycistronic transcription units (PTUs) raises fundamental questions about how endogenous RS is managed while preserving the remarkable genome plasticity that underpins parasite adaptation. To investigate the spatial organization of the replication stress response (RSR), we mapped the genome-wide distribution of the RSR markers γH2A and RPA1 in Leishmania major using ChIP-seq under physiological conditions, following acute replication fork stalling, and in parasites with impaired ATR function. Even in unperturbed cells, both markers accumulated preferentially at PTU boundaries, identifying these regions as constitutive RS hotspots. Following replication stress induction, RSR factors became enriched around early S-phase replication origins in a pattern consistent with bidirectional fork progression. Prolonged stress redistributed these signals into broader chromosomal domains centred on transcription initiation and, most prominently, transcription termination sites, indicating that these regions experience persistent endogenous replication stress. ATR-deficient parasites

displayed a marked expansion of RPA1- and γH2A-enriched regions, accompanied by widespread single-stranded DNA accumulation, demonstrating that ATR is required to constrain endogenous RS and coordinate replication with ongoing transcription. These findings identify PTU boundaries as major sites of endogenous replication stress and reveal that chromosome architecture plays a central role in defining where replication-associated challenges arise in the Leishmania genome. Our results support a model in which the ATR-dependent RSR continuously limits replication-associated DNA damage at these vulnerable chromosomal domains, thereby preserving genome integrity while maintaining the genomic plasticity required for parasite adaptation. More broadly, this work establishes a mechanistic framework linking genome organization, endogenous replication stress and genome maintenance in trypanosomatid parasites, providing new insights into how unconventional chromosome biology shapes both genome stability and adaptive evolution. **Keywords:** replication stress; genome maintenance; ATR; RPA1; γH2A; Leishmania.

RT.01 – 003 - **Genetic Diversity and Genome Plasticity in Trypanosomatids: Implications for Adaptation, Virulence, and Diagnosis**

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Genetic diversity and genome plasticity are hallmark features of trypanosomatid genomes. In *Trypanosoma cruzi* and Leishmania, copy number variations, aneuploidies, genome rearrangements, and single-nucleotide polymorphisms are common sources of genomic variation associated with drug resistance, environmental adaptation, parasite fitness, and virulence. Our group has investigated genomic instability during the experimental maintenance of *T. cruzi*, genomic diversity among Leishmania field isolates, and the implications of T. cruzi diversity for Chagas disease diagnosis. Experimental maintenance of *T. cruzi* revealed substantial genomic changes associated with altered parasite infectivity. Comparative analyses of parasites maintained under different host cells identified variation affecting multigene families encoding surface proteins involved in host-parasite interactions. These findings underscore the capacity of the *T. cruzi* genome for rapid remodeling and its potential contribution to parasite adaptation and virulence. Growing evidence indicates that *T. cruzi* genetic variability can affect diagnostic test performance. Conventional approaches based on assembled reference genomes capture only a fraction of the diversity circulating in endemic areas and may inadequately resolve repetitive regions and large multigene families encoding immunologically relevant proteins. We therefore developed an assembly-independent strategy based directly on sequencing reads from field isolates representing the six major discrete typing units (TcI–TcVI). Comparative k-mer analyses identified conserved sequences within highly variable surface protein families and peptides with promising serodiagnostic performance in samples from different endemic regions. This strategy highlights the value of naturally circulating isolates for identifying diagnostic targets that better represent *T. cruzi* diversity. Finally, genomic surveillance of Leishmania infantum field isolates from distinct visceral leishmaniasis-endemic areas in Brazil revealed marked spatial genetic diversity and shifts in circulating parasite genotypes within a two-year period. These findings indicate substantial spatiotemporal dynamics in parasite populations, reinforcing the importance of continuous genomic surveillance and highlighting challenges for disease control. Together, these studies illustrate how genome plasticity and genetic diversity operate across different biological scales, influencing parasite adaptation and virulence, population dynamics, and diagnostic performance. Understanding this genomic complexity is essential for developing more robust strategies for diagnosis, surveillance, and disease control. **Supported by:** CNPq, FAPEMIG, NMRC **Keywords:** Trypanosomatids; genome plasticity; genetic variability.

RT.01 – 004 - **From development to transmission: Understanding the development regulation of trypanosomes in the tsetse fly**

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Trypanosoma (T.) brucei, the causative agent of human African trypanosomiasis and nagana in livestock, undergoes a complex and tightly regulated series of developmental transitions within its tsetse fly vector. Following uptake from the mammalian bloodstream, parasites migrate and adapt to distinct tissue environments which include the midgut, proventriculus, and salivary glands. In the salivary glands, trypanosomes differentiate into mammal-infective metacyclic forms, poised for transmission into the next mammalian host. This progression

through at least nine discrete life-cycle stages in the fly is accompanied by extensive transcriptional reprogramming and sequential remodelling of the parasite surface coat, processes essential for immune evasion, tissue tropism, and the acquisition of infectivity. Despite the biological importance of these transitions, the regulatory networks that coordinate developmental progression and surface coat remodelling remain poorly understood. To address this, we are using CITE-seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing), to unravel these developmental transitions. CITE-seq enables simultaneous profiling of transcriptomic states and surface epitope expression at single-cell resolution, revealing previously hidden population heterogeneity and identifying candidate regulatory transitions during *T. brucei* development in the tsetse fly. By integrating single-cell RNA-seq with surface-protein data, we aim to move beyond descriptive associations and identify candidate regulators of stage-specific surface remodelling and the acquisition of mammalian infectivity. This integrated approach provides a framework for dissecting the gene-regulatory logic of vector-stage development.

RT 02 - Diversity, Ecology and Evolution of Protists

RT.02 – 002 - **Escaping Ice and Animals: Did Snowball Earth and the Rise of Metazoans Drive Testate Amoebae into Continental Waters?**

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The Neoproterozoic (1000–541 Ma) was a turning point in Earth's history, marked not only by the Cryogenian Snowball Earth glaciations but also by the ecological revolution associated with the emergence of animals. Vase-Shaped Microfossils (VSMs), interpreted as extinct marine testate amoebae mainly affiliated with Amoebozoa, provide the earliest fossil evidence of predatory protists and offer a unique opportunity to investigate how microbial eukaryotes responded to these two transformative events. Here, we integrate published taxonomic and taphonomic data with the South American fossil record to reconstruct the evolutionary history of VSMs across the Tonian, Cryogenian, and Ediacaran and propose a new scenario for the transition of testate amoebae from marine to continental freshwater environments. During the Tonian, VSMs reached their highest known diversity, with more than twenty species described worldwide in shallow marine microbial ecosystems. This flourishing biosphere collapsed during the Cryogenian glaciations, when diversity declined to only nine known species. Rather than representing a terminal extinction, this bottleneck may have selected lineages capable of surviving in low-salinity glacial refugia, including meltwater ponds, cryoconite holes, and protected coastal settings. We propose that adaptation to these environments constituted an osmophysiological pre-adaptation that later facilitated the colonization of continental freshwater habitats. Although climatic conditions improved after the Marinoan deglaciation, marine ecosystems were no longer the same. The Ediacaran fossil record preserves only six surviving VSM species, revealing a relict community that recovered in the presence of an entirely new ecological actor: benthic metazoans. Morphometric analyses indicate contrasting evolutionary responses during this interval, with *Cycliocyrillium simplex* exhibiting significant size reduction consistent with a possible Lilliput Effect, whereas *Bonniea dacruchares* and *Limeta lageniformis* retained remarkable morphological stability across tens of millions of years. At the same time, increasing bioturbation, substrate modification, grazing, and the expansion of animal-dominated benthic communities fundamentally altered the shallow-marine habitats in which VSMs had evolved for hundreds of millions of years. We hypothesize that the long term evolutionary shift of testate amoebae toward continental freshwater ecosystems was driven by the combined action of these two successive filters. Snowball Earth provided the physiological opportunity by selecting lineages tolerant of reduced salinity, whereas the rise of metazoans progressively diminished the ecological stability of their ancestral marine niche. Together, these processes reshaped the evolutionary trajectory of testate amoebae, ultimately favoring one of the most remarkable habitat transitions in protistan evolution. **Keywords:** Glaciations; protists; cryoconites.

RT.02 – 003 - **Diversity of microbial communities in aquatic and terrestrial environments**

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The use of the DNA metabarcoding technique has become very frequent in studies focusing on ecology and diversity of microbial communities at different environments. This technique enables generation and processing of large amounts of data in a much shorter time, keeping scientifically acceptable accuracy for the results and overcoming the need of different specialists to analyze the samples. It also accounts for the presence of rare species that inherently tend to be neglected in morphological studies. In my talk I will discuss the application of the DNA metabarcoding technique to investigate microbial communities in different environments, including bromeliad water, soil and aquatic polluted environments. **Keywords:** DNA metabarcoding; ciliates; 18s.

RT.02 – 004 - **A trait-based approach in ciliate functional ecology: toward a standardized framework**

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Ciliates represent a diverse and functionally important group of protists, displaying remarkable variation in morphology, behavior, feeding strategies, and life cycles. Through their roles as consumers, prey, and recyclers of organic matter, they contribute to carbon fluxes, nutrient cycling, and the regulation of ecosystem processes across aquatic and terrestrial environments. Despite their ecological relevance, efforts to characterize ciliate functional diversity have often relied on a limited set of traits or on classifications developed for specific habitats, taxonomic groups, or defined research questions. As a result, comparisons among studies remain difficult, hindering the development of broader ecological syntheses and general predictive frameworks. In this context, drawing on a comprehensive literature review and a meta-analysis conducted following PRISMA guidelines, we present a proposal for a unified terminology and standardized framework that organizes ciliate functional traits. To build this framework, the systematic literature search retained 69 studies, which were subsequently analyzed in RStudio to characterize research trends and patterns of keyword co-occurrence. The analysis revealed strong biases toward marine (31.5%) and freshwater (21.9%) systems, along with a concentration of research efforts in China and Brazil; however, with a marked underrepresentation of South American ecosystems. Most studies focused on habitat affinity and feeding strategies, whereas morphological, behavioral, and trophic-mode traits have received comparatively less attention. Furthermore, the identification of 16 thematic communities suggested that trait-based research in ciliates remains fragmented rather than unified. Building on this synthesis, ciliate functional traits were organized and codified in five major dimensions: (1) morphological traits, (2) trophic mode and metabolic strategy, (3) feeding strategy and trophic interactions, (4) behavioral and life-history traits, and (5) habitat affinity and environmental preferences. Beyond describing these categories, the analysis synthesizes current knowledge to explore how their integration can improve our understanding of community assembly, species coexistence, ecosystem functioning, and ecological responses to environmental change. **Keywords:** Ciliophora; functional traits; standardized trait-codification.

RT 03 - Vectors

RT.03 – 001 - **Effect of Anopheles midgut microbiota bacteria on the sexual development of *Plasmodium vivax* in *Anopheles darlingi***

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Malaria remains a major public health challenge worldwide, particularly in tropical and subtropical regions where transmission is sustained by Anopheles mosquitoes infected with Plasmodium parasites. Colombia reports one of the highest malaria burdens in the Americas, yet current control strategies based on insecticides and antimalarial drugs are increasingly compromised by the emergence of insecticide-resistant mosquito populations and drug-resistant parasites. Furthermore, malaria vaccination has not been implemented as a control strategy in Colombia and Brazil, highlighting the need for innovative approaches to reduce disease transmission. The mosquito gut microbiota has emerged as a promising target for malaria control because of its capacity to influence parasite development and vector competence. Studies in Africa and Asia have identified bacterial taxa, including Enterobacter and Serratia marcescens, capable of inhibiting Plasmodium falciparum through mechanisms such as reactive oxygen species production, immune modulation, nutrient competition, and secretion of antiparasitic compounds. However, the relationship between mosquito-associated bacteria and Plasmodium is highly complex and appears to vary according to mosquito species, parasite species, and ecological context. For example, in the neotropical vector Anopheles (Nyssorhynchus) darlingi, the descrease of gut microbiota did not affect Plasmodium vivax infection, contrasting with previous observations in P. falciparum and suggesting that microbiota–parasite interactions are species-specific. In South America, several studies have characterized the gut microbiota of Anopheles vectors, including the principal Colombian malaria vectors Anopheles (Nyssorhynchus) albimanus, Anopheles (Nyssorhynchus) nuneztovari, and Anopheles (Nyssorhynchus) darlingi, using both culture-dependent and high-throughput sequencing approaches. Despite these advances, little is known about the functional role of bacterial isolates from Colombian mosquito populations in modulating parasite development or vector competence. Investigating these interactions may identify native bacterial candidates with antiparasitic properties and contribute to the development of microbiota-based strategies for malaria control. Such approaches could complement existing interventions and provide sustainable alternatives to address the growing challenges of insecticide and drug resistance. Premilinar data demonstrates that the cultivable gut microbiota of An. darlingi is dominated by Proteobacteria, a bacterial group with diverse biochemical capabilities that may shape mosquito–parasite interactions. Three bacterial isolates significantly inhibited P. vivax development by reducing oocyst numbers, while bacterial supernatant exhibited inhibitory activity, particularly against the sporozoite stage. These findings identify promising bacterial candidates and their bioactive compounds for the development of microbiota-based approaches aimed at reducing malaria transmission. Future studies integrating genomic, metabolomic, and functional analyses will be essential to identify the mechanisms underlying these inhibitory effects and to evaluate their potential for vector control. **Keywords:** mosquito microbiota; Plasmodium; vector competence.

RT.03 – 002 - **Vitellogenin Receptor Function Shapes Fertility and Microbial Inheritance in *Rhodnius prolixus***

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The vitellogenin receptor (VgR) mediates yolk protein uptake during follicle development and is essential for embryogenesis in oviparous species. Here, we characterize the single *Rhodnius prolixus* VgR isoform and uncover an unexpected role in microbial regulation within the reproductive system. The receptor displays a conserved LDLR-like structure and is highly expressed in early oocytes. RNAi-mediated VgR silencing caused defective yolk granule biogenesis, leading to the accumulation of the main yolk protein precursors, Vg and RHBP, in the hemolymph, yet oviposition and fertilization proceeded normally. The resulting eggs were yolk-depleted and non-viable. Remarkably, VgR knockdown reduced bacterial 16S rRNA levels in oocytes while

promoting the expansion of several members of the core virome, a phenotype not reproduced by Vg silencing. Neither purified Vg nor changes in immune (defensin) or RNA interference pathways explained the microbial shifts. These findings indicate that VgR influences not only yolk endocytosis but is also associated with changes in microbial composition within developing oocytes. We propose that VgR may contribute to a functional link between yolk endocytic dynamics and microbial homeostasis, thereby affecting the balance of microbial components in the oocyte. This connection broadens the functional context of VgR and provides new insight into how vertical transmission processes may be shaped in this major Chagas disease vector. **Keywords:** Vitellogenin receptor, Yolk uptake; Microbiota homeostasis, Oogenesis; Chagas disease vector, Vertical transmission.

RT.03 – 003 - **Trypanosomatids from the Brazilian Amazon: Invertebrate (dipterans) and vertebrate (didelphimorphs, rodents, anurans, squamates) hosts**

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Protozoa of the family Trypanosomatidae are obligate parasites of vertebrates, invertebrates, and plants. They exhibit great biological diversity; to date, 19 monoxenous genera (infecting insects, primarily from the orders Diptera, Hymenoptera, Siphonaptera, and Hemiptera) and 5 heteroxenous genera (infecting plants or vertebrates such as birds, reptiles, amphibians, and mammals) have been described. These parasites are best known for the role certain species play in diseases such as Chagas disease and leishmaniasis; however, there remains a significant gap in our knowledge of this group. The primary objective of this study was to investigate the diversity of trypanosomatids and their vertebrate and invertebrate hosts in the Amazon region. Field sampling was conducted in forest and peri-urban environments within the municipalities of Urucurituba and Presidente Figueiredo, Amazonas (AM). Small vertebrates belonging to the orders Anura, Squamata, Rodentia, and Didelphimorphia were collected, as well as insects of the order Diptera (specifically Culicidae/mosquitoes, Ceratopogonidae/biting midges, and Psychodidae/sand flies). Blood and liver samples were collected from the vertebrates; slides were prepared, cultures were inoculated, DNA was extracted, and PCR and sequencing were performed. Insect intestines were dissected and placed in culture media. A total of 68 anurans and 73 reptiles were examined; infection rates via thick blood smears were 4.36% and 0%, respectively, while PCR detection rates were 7.3% and 0%, respectively, with sequences showing similarity to *Trypanosoma spp.* Additionally, 42 didelphimorphs and 64 rodents were sampled; no parasites were observed in thick blood smears, but PCR analysis revealed infection rates of 2.38% and 6.25%, respectively, with sequences resembling *Trypanosoma sp.* and *Trypanosoma cruzi. Zelonia spp.* and *Strigomonas galati* were isolated and identified in biting midges; *Crithidia otogatchiensis*, *Crithidia confusa*, *Crithidia melifica*, and *Leptomonas pyrrhocoris* in mosquitoes; and *Crithidia otogatchiensis*, *Endotrypanum sp*., and *Trypanosoma sp.* in sand flies. Although analyses are not yet complete, the results obtained so far show a high rate of trypanosomatid infection in anurans and small rodents, with no recorded infections in reptiles. The diversity of monoxenous trypanosomatids in the analyzed sample groups was high; however, further analyses will help determine infection rates and the host specificity of these parasites. **Keywords:** Biodiversity; Amazon region; Trypanosomatidae hosts.

RT.03 – 004 - **Sand fly immunity and gut receptors - implications for Leishmania infection in the vector**

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Phlebotomine sand flies are vectors of Leishmania parasites and other medically important pathogens. Successful parasite infection depends on a complex series of interactions within the insect midgut, including the vector immune response. Significant advances have been made in understanding the molecular dialogue between Leishmania and its vectors; nevertheless, the mechanisms coordinating parasite recognition and immune regulation in sand flies remain underexplored. Current knowledge of sand fly innate immunity, mainly derived from functional studies, indicates that conserved immune pathways regulate microbial homeostasis and can influence vector competence. The Toll, IMD, and JAK/STAT pathways, together with effector molecules such as antimicrobial peptides, contribute to the regulation of gut microbiota and Leishmania infection. Experimental evidence demonstrating that modulation of immune signaling affects both parasite survival and bacterial abundance reinforces the importance of sand fly innate immunity in infection establishment. Our studies on Lutzomyia longipalpis and Phlebotomus papatasi have revealed that parasite-derived molecules can influence vector immune responses. Surface glycoconjugates such as lipophosphoglycan (LPG) from Leishmania major and Leishmania infantum, which contribute to parasite survival and midgut colonization, also modulate the expression of antimicrobial peptides in these sand flies. In parallel, Gram-negative bacteria present in the sand fly gut expose lipopolysaccharides (LPS), a well-characterized pathogen-associated molecular pattern (PAMP). Our results showed that both LPG and LPS can be recognized by the insect immune system and trigger changes in gene expression. These findings support the concept that parasite development results from a dynamic molecular crosstalk involving the parasite, gut microbiota, and vector immune system rather than from passive adaptation to the insect gut environment. In addition, we are investigating the role of sand fly midgut receptors in microbial recognition and immune modulation. Our data indicate that a mucin-like protein and a gut-specific galectin, previously characterized as receptors involved in parasite attachment to the gut of *L. longipalpis* and *P. papatasi*, respectively, may also participate in the initial response to Leishmania and bacteria. Exposure of sand flies to LPG or LPS influences the expression of both receptor genes. For example, *L. infantum* LPG and Escherichia coli LPS upregulate the mucin-like protein in *L. longipalpis*. Similarly, L. major LPG increases galectin expression in *L. longipalpis*, whereas E. coli LPS induces galectin expression in *P. papatasi*. These observations suggest that receptors traditionally associated with parasite adhesion may also be regulated by microbial stimuli, raising new hypotheses regarding their participation in immune sensing and the physiological interactions between sand flies and their gut-associated microorganisms. Gene function studies using RNAi-mediated gene silencing can address whether the suppression of these gut receptors interferes with Leishmania establishment and sand fly immune responses. The integration of classical immune pathways with receptor-mediated microbial recognition offers new perspectives on how sand flies discriminate among microorganisms while maintaining gut homeostasis and supporting or restricting parasite establishment.
Supported by: This study was supported by the Czech Science Foundation (GACR 25-15318S)
Keywords: Sand fly immunity; Leishmania; Midgut receptors.

RT.04 – Drivers of Diversity: Kinases, Translation, and the Evolution of Cell Populations

RT.04 – 001 - **Translational tricks and trypanosome tactics**

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The translational machinery is highly conserved across eukaryotes, but trypanosomatid parasites have evolved several unusual mechanisms that expand our understanding of translation. Several of these involve changes in tRNA function, including altered codon recognition and post-transcriptional tRNA modification. In Blastocrithidia nonstop, specialized tRNAs have enabled the reassignment of all three canonical stop codons to encode amino acids, while UAA retains a dual role as both a sense codon and a translation termination signal. Unlike other stop-codon-reassigned lineages, such as ciliates, B. nonstop transcripts contain unusually long 3′ UTRs, raising the question of how ribosomes distinguish between coding and terminating UAAs. Using ribosome profiling, proteomics, and Oxford Nanopore direct RNA sequencing, we found that most proteins terminate at the annotated UAA stop codon, although a subset undergoes stop codon readthrough. C-terminally extended proteins, together with the enrichment of UAA codons in 3′ UTRs, support a model in which multiple downstream UAA codons contribute to efficient, context-dependent translation termination. In Leishmania mexicana, we investigated how the nutrient-derived post-transcriptional tRNA modification queuosine (Q) contributes to translational adaptation during differentiation between the insect and mammalian stages of the parasite. Although queuosine is dispensable for parasite growth and differentiation under standard laboratory conditions, it promotes efficient decoding of Q-tRNA-dependent codons, resulting in codon-biased changes in protein expression. Loss of queuosine reduces parasite survival in macrophages and attenuates virulence in mice, demonstrating the importance of tRNA modification for adaptation to the mammalian host. Together, these findings reveal diverse adaptations of the translational machinery in trypanosomatid parasites. More broadly, our work demonstrates how evolutionarily divergent organisms can reveal fundamental principles of translation that remain hidden in conventional model systems. **Keywords:** translation; tRNA; trypanosomatids.

RT.04 – 002 - **Gene expression programs driving Leishmania differentiation and transmission in the sand fly**

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Successful transmission of Leishmania parasites depends on their ability to complete a series of developmental transitions within the sand fly gut. The gene expression programs underlying these transitions have been difficult to define using bulk sequencing, which averages signals across mixed parasite populations and offers no validated markers for isolating individual stages. We used single-cell RNA-sequencing to chart the transcriptional landscape of Leishmania major as it colonizes its natural vector, *Phlebotomus duboscqi*, sampling midgut infections at early (day 2), intermediate (day 5), and late (day 9) time points, as well as parasites present in the transmitted inoculum. This approach resolved distinct gene expression signatures for each developmental subpopulation and yielded molecular markers that we validated *in vivo* through endogenous fluorescent tagging. Reconstructing developmental trajectories from these data showed that differentiation does not follow a simple linear sequence. Instead, after reaching the leptomonad stage, parasites follow one of two paths: they either differentiate into haptomonads, which attach to or detach from the gut and stomodeal valve, or enter a previously unrecognized dividing early-metacyclic state. These early-metacyclics most likely mature into the classical late-metacyclic form highly motile, non-dividing, and highly infectious. Profiling the parasites egested during a bite further revealed that detached haptomonads are transmitted together with late-metacyclics, rather than being confined to the midgut. Using fluorescence-activated cell sorting to isolate these subpopulations, we found that haptomonads are themselves infectious in the mammalian host and can worsen disease outcome. Ongoing efforts are focused on spatial transcriptomic analysis of infected midguts at various infection time points to investigate both fly and parasite gene expression patterns in situ. Together, these results establish a molecularly defined, stage-resolved map of Leishmania development in the sand fly and identify the gene expression programs that may govern parasite differentiation, vector colonization, and transmission to the mammalian host. **Keywords:** single-cell RNA-seq; Leishmania; sand fly.

RT.04 – 003 - **Cell Cycle Regulation in *Trypanosoma cruzi*: Conserved Kinases with Parasite-Specific Functions**

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Cell-cycle progression is controlled by conserved cyclin-dependent kinase (CDK)-cyclin complexes whose activities are regulated by cyclins and inhibitory phosphorylation. In Trypanosoma cruzi, however, the mechanisms governing cell-cycle transitions remain poorly understood. We investigated the functions of the cell-cycle kinases CRK1 and CRK3 and the inhibitory kinase Wee1 using CRISPR/Cas9-mediated genome editing to determine their roles in cell-cycle progression and benznidazole susceptibility. CRK1 and CRK3 displayed relatively constant expression throughout the cell cycle, as expected for CDKs, but exhibited distinct subcellular localization. CRK3 localized predominantly to the nucleus and was also associated with kinetoplast DNA during its segregation, suggesting a role in coordinating nuclear and mitochondrial genome inheritance. In contrast, CRK1 localized exclusively to cytoplasmic structures that colocalized with the mitochondrion, indicating a divergent function. Wee1 showed a conserved oscillatory expression profile, peaking during S/G2 phases, localized to the nucleus except during mitosis, and phosphorylated tyrosine residues in CRKs, consistent with its inhibitory activity. Wee1 knockout parasites were viable but displayed defective cell division, increased frequencies of zoids, and enhanced susceptibility to benznidazole, linking cell-cycle regulation to drug response. In contrast, CRK1/CRK3 double-knockout parasites could not be obtained, suggesting that their combined activity is essential for parasite viability. CRK1 single-knockout parasites showed no detectable cell-cycle defects but exhibited elongated cell bodies and shorter flagella, revealing a role in morphogenesis. Together, these findings demonstrate that conserved cell-cycle regulators have acquired parasite-specific functions in T. cruzi, coordinating cell division, morphogenesis, mitochondrial dynamics, and susceptibility to chemotherapy. **Keywords:** Cell cycle; kinases; drug resistance.

RT.04 – 004 - **The NEK family in *Trypanosoma cruzi*: focus on RDK2 in differentiation and host–parasite interaction**

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Trypanosoma cruzi, the etiological agent of Chagas disease, undergoes complex developmental transitions that are essential for parasite survival, transmission, and host infection. In trypanosomatids, protein phosphorylation is a central regulatory mechanism, and NIMA-related kinases (NEKs) have been implicated in morphogenesis, basal body duplication, kinetoplast segregation, differentiation, and cytokinesis. Comparative kinome analyses indicate that *T. cruzi* harbors an expanded and divergent NEK repertoire, reinforcing the idea that this family may play important and specialized roles in parasite biology. Our research is aimed at understanding the contribution of the NEK family to *T. cruzi* differentiation and host–parasite interaction, using TcRDK2 as a starting point for functional characterization. TcRDK2 is a conserved NEK kinase expressed across the parasite life cycle, and our findings indicate that it is associated with processes linked to developmental progression, cell division-related events, and mammalian cell infection. In addition, phosphoregulation analyses suggest that TcRDK2 may participate in broader signaling networks involving cytoskeletal organization and translation-associated pathways. Taken together, these results support the NEK family as an important and still underexplored regulatory hub in *T. cruzi.* By focusing on RDK2 while expanding our interest to other NEK kinases, we seek to define how this family contributes to parasite adaptation, differentiation, and infectivity. This work may help reveal kinetoplastid-specific regulatory mechanisms and support the identification of new candidate targets for Chagas disease intervention.

RT.05 – Immunity to Trypanosoma cruzi in the Americas

RT.05 – 001 - **How *Trypanosoma cruzi* Diversity Shapes Human Immune Responses**

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Trypanosoma cruzi is a genetically diverse parasite, comprising six discrete typing units (DTUs), TcI to TcVI, which differ in their geographical distribution, biological characteristics, and interactions with the host. This diversity may contribute to the remarkable heterogeneity observed in the clinical manifestations and outcomes of Chagas disease. In this lecture, I will discuss how parasite diversity shapes host–pathogen interactions, with a particular focus on the early immune response to distinct T. cruzi strains. Experimental studies comparing strains belonging to different DTUs have revealed that genetically distinct parasites can trigger markedly different immune profiles. In human monocytes, for example, distinct strains can induce opposing inflammatory or regulatory responses, characterized by differences in cytokine production and cellular activation. Interestingly, simultaneous exposure to immunologically divergent strains can further modify these responses, resulting in enhanced monocyte activation and increased production of both inflammatory and regulatory cytokines. I will discuss how these strain-specific interactions may influence parasite control, persistence, and the subsequent development of Chagas disease. Particular attention will be given to the concept that different T. cruzi populations may establish distinct immunological environments during the early stages of infection, potentially contributing to differences in disease progression and clinical manifestations. Understanding these interactions may also help explain why individuals infected with genetically distinct parasites can experience markedly different disease outcomes.

RT.05 – 002 - **From Fundamental Research to Public Health: Congenital Chagas Disease in Bolivia**

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Human infection with *T. cruzi* poses a challenge to the immune system, which must cope with a parasite that adopts three different forms during the course of infection: metacyclic trypomastigote, amastigote, and bloodstream trypomastigote. This interaction between the immune response and the parasite is key to the evolution of the infection. Congenital Chagas disease occurs when *T. cruzi* crosses the placental barrier during pregnancy or delivery, and the newborn is born infected. Maternal *T. cruzi* infection has immunological implications for both the mother and the newborn, even in the absence of congenital infection.[1] *T. cruzi* infection in mothers induces a potent state of cell activation in uninfected neonates (and probably in fetuses), leading to the simultaneous production of pro- and anti-inflammatory citokines in the presence of parasite antigens.[2,3] Such maternal influence on neonatal innate immunity might have protective effects, limiting the occurrence and the outcome of congenital infection.[4] In addition, congenitally infected newborns show a strong, adult-like CD8 T-cell response,[5,6] an activation of fetal γδ T cells,[7] and phenotypic and functional modifications of cord blood NK cells;[8] this specific immune activation persists during the first months of life and is accompanied by a trend toward a Type-1 response to vaccines in infants.[9] Beyond these immunological changes, it is crucial to point out that the treatment of newborns congenitally infected with *T. cruzi* before 12 months of age achieves cure rates close to 100%. These data were decisive and provided the basis for the government to launch the Bolivian national control program for congenital Chagas disease. In 2004, a pilot project introduced universal screening of pregnant women in Bolivia, together with the follow-up of newborns of *T. cruzi*-infected mothers, with the aim of treating the infants when positive.[10] In 2011, the Bolivian National Chagas Program incorporated the guidelines for the diagnosis and treatment of congenital Chagas disease into its own regulations, to be applied at the national level.[11] This model is a good example of how basic research can guide public health policies.

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RT.05 – 003 - **Cellular and Molecular Biomarkers of Cardiac Remodeling: Insights from the Brazilian Scenario of Chagas Cardiomyopathy**

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Chronic Chagas cardiomyopathy (CCC) represents the most severe manifestation of Chagas disease, affecting approximately 30% of infected individuals, whereas the majority remain in the asymptomatic Indeterminate (IND) clinical form. A central question is why some infected individuals remain in the indeterminate clinical form while others progress to chronic Chagas cardiomyopathy. Understanding the immunological mechanisms that prevent or drive the progression from IND to CCC is critical for identifying prognostic biomarkers and therapeutic targets. In the Brazilian scenario, extensive immunopathopathological characterization reveals that the clinical course of chronic Trypanosoma cruzi infection is finely orchestrated by a delicate balance between active immunoregulation, systemic cytokine networks, and extracellular matrix (ECM) remodeling dynamics. Our group demonstrated that the maintenance of the IND form relies on an effective mmunoregulatory network. CD4+ CD25high FOXP3+ regulatory T cells (Tregs) from IND patients preserve their functional capacity to suppress effector cell responses and proinflammatory cytokine secretion. This process is reinforced by specific FOXP3 gene polymorphisms associated with the asymptomatic clinical status. Furthermore, fine-tuning of regulatory T-cell responses is mediated by key costimulatory signaling pathways, particularly via CD80 and CD86 interactions with CD28 and CTLA-4, which actively dampen an excessive immune activation in IND individuals. Additionally, monocyte and neutrophil populations in IND patients display an overall modulatory cytokine profile, characterized by downregulation of phagocytosis-related surface receptors, differential IL-1β activation pathways, and regulated apoptosis mechanisms, preventing tissue damage. On the other hand, the loss of these immunoregulatory mechanisms leads to persistent systemic inflammation and cardiac tissue damage. While protective immune responses, such as elevated IL-17 production correlate with preserved left ventricular function in IND patients, systemic cytokine imbalance drives chronic leukocyte activation. Activated circulating monocytes and neutrophils become major sources of matrix metalloproteinases 2 (MMP-2) and 9 (MMP-9). The differential expression and release of MMP-2 and MMP-9 in CCC patients lead to progressive ECM degradation, microvascular changes, and myocardial fibrosis. Elevated plasma levels of MMP-2 and MMP-9 strongly correlate with cardiac morbidity and serve as reliable predictive biomarkers for clinical progression from the indeterminate to the cardiac form. Taken together, the Brazilian clinical scenario demonstrates that chronic Chagas disease pathogenesis is governed by a transition from an actively regulated network, mediated by functional Tregs, costimulatory regulation, and protective cytokines to a dysregulated inflammatory phenotype driven by MMP overexpression and matrix remodeling. These molecular and cellular signatures provide key insights into host-parasite interactions in the Americas and highlight promising biomarkers for early clinical stratification and targeted therapeutic interventions. **Supported by:** CNPq and FAPEMIG
Keywords: Biomarkers; Cardiac Remodeling; Immunoregulation.

RT.06 – One Health

RT.06 – 001 - **Detection of silent circulation of parasites in ecoepidemiology investigations from a One Health perspective**

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Silent circulation of parasites represents one of the most challenging aspects of ecoepidemiology. These are situations where parasites persist in ecosystems without producing obvious clinical cases, quietly moving between wildlife, domestic animals, and sometimes humans. Because infections may remain asymptomatic or unnoticed, transmission continues beneath the radar, creating hidden risks for health and biodiversity. Ecoepidemiology provides the tools to uncover this invisible dynamic. By combining epidemiology, ecology, and parasitology, we can investigate reservoirs, vectors, and environmental conditions that sustain parasites. Surveillance of wildlife and domestic animals using sensitive tools, and genetic investigation, through employment of molecular diagnostics including PCR and sequencing, are essential to observe the parasites silent circulation. Serological studies also reveal past exposures, helping us understand the true extent of parasite presence. Examples illustrate the importance of this approach. Cryptosporidium can circulate silently in water sources, spreading through asymptomatic carriers. Leishmania and Trypanosoma often persists in wild reservoirs before human outbreaks occur. Toxoplasma gondii silently circulates in ecosystems, affecting wildlife health and posing zoonotic risks. Each case shows how parasites can remain hidden until conditions trigger visible disease. From a One Health perspective, silent circulation has broad implications. For humans, it means outbreaks may emerge suddenly without warning. For animals, it can reduce productivity in livestock and threaten wildlife conservation. For the environment, it disrupts ecological balance and resilience. Addressing these challenges requires interdisciplinary collaboration, integrating veterinary, medical, and environmental sciences. With the aim of producing ecoepidemiological studies in landscapes with different patterns of degradation, our research group conducted an investigation of various pathogenic protozoa in human population, animals, and arthropod vectors at the rural settlement of Santa Luzia, located in Cruzeiro do Sul, Acre, in the Brazilian Amazon region. To carry out these studies, we employed molecular diagnosis through PCR and genetic studies using markers developed by our research group, which demonstrate sensitivity and specificity for detecting species of Trypanosoma and Leishmania. The results revealed the circulation of Trypanosoma cruzi and Trypanosoma rangeli in triatomines colonizing palm trees. In humans and domestic animals, we observed the circulation of T. cruzi with genetic characteristics distinct from those found in triatomines in the same areas, indicating the silent circulation of undescribed genetic strains of T. cruzi in the region. Notably, we recorded an increase in the incidence of chronic Chagas disease in its indeterminate phase in the human population over a four-year period. Additionally, we detected the silent circulation of Leishmania infantum in dogs, a species responsible for visceral leishmaniasis, which can cause significant morbidity and mortality in affected populations. The detection of T. cruzi by serologic and molecular tests in animals used for meat and milk production has raised concerns about the risk of Chagas disease transmission through the consumption of animal-derived products in the region. This finding highlights the need to deepen investigations into this disease in the area. By detecting silent circulation early, we can prevent outbreaks, protect ecosystems, and safeguard health across species. In conclusion, silent circulation is a hidden but critical threat. Ecoepidemiology allows us to reveal it, and One Health strategies provides the framework to act upon it. Together, they form the path toward a healthier and more resilient coexistence between humans, animals, and the environment. **Keywords:** Ecoepidemiology; Silent circulation; Kinetoplastidae.

RT.06 – 002 - **Insights into trypanosome infection biology from the bovine model**

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The study of trypanosome infection biology is dominated by the mouse model. For African trypanosomes, this tractable experimental system has enabled us to build substantial understanding of facets of trypanosome phenotypes that enable establishment and persistence, and of aspects of the host immune response that are critical to control or susceptibility to infection. Studies are also dominated by Trypanosoma brucei, where we have an advanced understanding of trypanosome molecular mechanisms that shape disease kinetics and outcome. However, most disease occurs in large ruminants, principally cattle, in which disease is caused by several genotypically and phenotypically divergent species of trypanosome – as well as *T. brucei*, *T. congolense* and *T. vivax* are implicated. We have spent the past decade (with collaborators) establishing capabilities and experimental models for T. congolense and T. vivax, and the bovine host. While clearly important for potential translation as the disease-relevant pathogens and hosts, the nature of the very chronic infections in cattle also means that studying infections in this host enables fundamental insights into stages of infection that are simply not possible in mice, where duration of infections is much more limited – how African trypanosomes achieve long term persistence (up to many 100s of days in cattle) remains a key question. Additionally, due to the relatively large size of cattle, it is possible to sample serially during long term infections, enabling longitudinal analysis at a resolution that is not possible in mice. We have undertaken analyses of key phenotypes during infections in cattle (trypanosome differentiation and antigenic variation, and the host immune response), which have revealed significantly novel insights into trypanosome infection biology, and which challenge several paradigms established in mice. This talk will summarise these advances enabled by the bovine model, and highlight current gaps in our knowledge that could further be addressed by studying trypanosomes in cattle.
Keywords: African trypanosome, African animal trypanosomiasi; antigenic variation, differentiation; immune response.

RT.06 – 003 - **Zoonotic Sarcocystidae: challenges for One Health in protozoology**

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The family Sarcocystidae comprises apicomplexan protozoans with complex life cycles, many zoonotic and posing challenges for public health, veterinary medicine, and wildlife conservation. *Toxoplasma gondii* stands out as a widespread opportunistic pathogen infecting virtually all warm-blooded animals, including humans. Sarcocystis species are also zoonotic, associated with waterborne and foodborne diseases, while Neospora, though not zoonotic, is a major agribusiness pathogen causing abortion and encephalitis in cattle and horses. In the One Health context, these protozoans exemplify the interconnection between human, animal, and environmental health. Transmission involves ingestion of oocysts from contaminated water/soil, consumption of undercooked meat with tissue cysts, and congenital transmission, demanding integrated surveillance. Climate change, land-use shifts, and urbanization have altered host distribution and environmental contamination patterns. This presentation reviews zoonotic Sarcocystidae, emphasizing *T. gondii* as a model pathogen and addressing advances in molecular epidemiology, genotyping, and population genetics that elucidated transmission dynamics and virulence. We also explore livestock management, food production, and human exposure risks, highlighting interdisciplinary approaches and behavioral changes in parasitized hosts. Our group has investigated Sarcocystidae in Brazil. We performed the first isolation and genotyping of *T. gondii* from bats in São Paulo, obtaining two isolates (TgBatBr1 from Molossus molossus and TgBatBr2 from *Desmodus rotundus*) with 100% mouse mortality and genotypes previously found in cats, chickens,

capybaras, sheep, and rabbits, suggesting a common Brazilian lineage. We later found wide genetic diversity of Sarcocystidae in bat tissues, reinforcing their role as sentinels and reservoirs. Recently, we have investigated *T. gondii*-induced behavioral changes in Rattus norvegicus, exploring gene expression of tissue receptors, the gut-brain axis, and behavioral correlations, providing insights into host-parasite neurobiological interactions. Environmental One Health has also been a focus. We participated in the REMONAR MCTI network, monitoring SARS-CoV-2 in wastewater, which reinforced the potential of wastewater-based epidemiology for detecting pathogens with environmental routes, including protozoan oocysts recently investigated in São Paulo's Guarapiranga reservoir. Integrating molecular epidemiology, environmental monitoring, and host-pathogen studies is essential for understanding Sarcocystidae transmission cycles. Emerging challenges include detecting atypical, potentially hypervirulent *T. gondii* genotypes in South America, linked to severe outcomes in immunocompromised patients, and growing evidence of environmental oocyst contamination in urban/rural areas. Wildlife's role as reservoirs/sentinels must be further explored, as shown by our studies involving humans, domestic/wild animals, and arthropod vectors. Collaborative networks among parasitologists, ecologists, veterinarians, physicians, and public health professionals are crucial. Future perspectives include developing diagnostic tools based on recombinant proteins and molecular approaches. Genomic surveillance, geospatial analysis, and community education are essential to reduce neglected protozooses burden. Only through interdisciplinary and transdisciplinary efforts can we effectively confront zoonotic Sarcocystidae challenges, safeguarding human, animal, and environmental health in a true One Health approach. **Keywords:** Toxoplasma gondii; foodborne protozoan; molecular epidemiology.

RT.06 – 004 - **The Fly That Shaped a Continent: African Trypanosomosis and the Evolution of One Health Solutions**

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African trypanosomosis is commonly viewed through the lens of disease control, yet its influence extends far beyond human and animal health. For centuries, the distribution of tsetse flies across sub-Saharan Africa shaped patterns of livestock production, human settlement, land use, and biodiversity conservation. Extensive regions unsuitable for cattle production due to trypanosome transmission remained comparatively undisturbed, helping to preserve unique ecosystems and influencing the social and economic development of the continent. In this sense, African trypanosomosis represents one of the most striking examples of how a vector-borne disease can influence the interconnected health of humans, animals, and the environment. Today, Africa is undergoing rapid demographic growth, agricultural expansion, and environmental change. Human populations are increasing, demand for livestock-derived foods is rising, and livestock production systems are expanding into new areas. These transitions are occurring at the interface of wildlife, livestock, and human communities, where the risks and consequences of vector-borne and parasitic diseases are most keenly felt. At the same time, advances in vector control, diagnostics, therapeutics, and surveillance are creating new opportunities to reduce the burden of trypanosomosis and other parasitic diseases. However, disease control alone is not sufficient. Sustainable livestock development requires consideration of broader One Health outcomes, including environmental protection, biodiversity conservation, food safety, drug residues, and the emergence of resistance. Future interventions must therefore be designed not only to improve efficacy against parasites and vectors, but also to minimise unintended impacts on ecosystems and safeguard human health. Using African trypanosomosis as a case study, this presentation explores how historical interactions between disease, people, livestock, and landscapes continue to shape contemporary challenges and opportunities. It argues that the next generation of parasite and vector control strategies should be evaluated through a One Health framework that balances food security, rural livelihoods, ecosystem integrity, and public health. In doing so, Africa has an opportunity to pioneer sustainable approaches to parasite control that support healthy animals, healthy people, and healthy environments. **Keywords:** Trypanosomosis; Africa; History.