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on the Biology and Pathology of Free-Living Amoebae

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Oral Presentations

Session I: ecology, genomes and diversity of free-living amoebae

Chairpersons: Vincent Delafont - Jacob Lorenzo Morales

Keynote speaker: Stephen Geisen (Wageningen, The Netherlands)

The distribution and ecology of soil protists with a focus on amoebae

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Keywords: Soil protists, Soil biodiversity, Ecosystem functioning

Soils are the basis for life on Earth as, for example, soils are major carbon sinks and provide virtually all of our food. Biodiversity studies in soils have, however, focused mostly on the ecology of bacteria and fungi in response to the surrounding abiotic parameters and plants. Despite the knowledge on the importance of predators as drivers of animal populations in macroscopic systems, we have generally little understanding about microbiome predators, particularly amoeboid protists, in soils.

I will provide an overview of the key microbiome predators: protists. I will show recent insights into the biogeographic distribution of protist communities in soils. This descriptive work provides the basis to better understand the functional importance of protists, which I will show with several examples of protists as puppet masters of soil microbiomes such as in catalyzing carbon and nutrient cycling and controlling plant performance. I will also show the importance of the immense diversity of protists for soil functioning as most previous knowledge has focused on few model species like from the genus *Acanthamoeba*, which often might not be representative for all protists in soils.

Together, I aim to provide a holistic overview of soil protists that should stimulate interactions among scientists working on amoeba with those in other scientific fields. I believe that we need this synergistic power to increase our knowledge on how Earth as a whole works, to combat many of the ongoing changes that threaten life on Earth.

O1.1

Comparative study of the mitochondrial genomes of *Acanthamoeba*

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Keywords: Mitochondrial genome; Sequence Types; genetic variation

With respect to DNA analysis, members of *Acanthamoeba* are among the most extensively sampled members within the Amoebozoa. Over 74,000 DNA sequences attributed to more than 6000 *Acanthamoeba* isolates have been deposited. Despite this abundance of molecular data, our ability to assign a new isolate to a particular "species" of *Acanthamoeba* remains tenuous, at best. How variable are the genes within *Acanthamoeba*? What patterns of diversification occur for *Acanthamoeba*? Examinations of nuclear 18S rRNA sequences (*Rns*) in the DNA databases indicate that *Acanthamoeba* contains at least 23 sequence clusters, "Sequence Types", which may correspond to species. Despite availability of more than 30 whole genome sequencing (WGS) projects in *Acanthamoeba*, comparisons among isolates using alternative genes to *Rns* are limited. To better elucidate questions about genetic diversity within *Acanthamoeba*, complete mitochondrial genomes of 33 isolates of *Acanthamoeba* have been extracted from the DNA databases. These include 23 T4 isolates and two T5 isolates. Sequences exist from a further seven Sequence Types (T2, T3, T7, T10, T18, T21 and T22). While these represent only 9 of the 23 Sequence Types, they provide a useful first analysis of mitochondrial variation. In general, mt-genome phylogenetic relationships parallel patterns from *Rns* sequences. *Acanthamoeba* mitochondrial genomes range in size from ~39,000 to >43,000 bases, and contain ~39 protein coding genes, 3 rRNA genes and ~15 tRNA genes. Mt-genome structure shows minor variation in both gene order and in the number of protein and tRNA genes, especially differentiating morphological Group 1 of *Acanthamoeba*, exemplified by *A. astronyxis*, when compared to members of morphological Groups 2 or 3. To assess how mitochondrial variation of *Acanthamoeba* compares to nuclear gene divergence, a small random sample of nuclear-encoded genes including some nuclear-encoded ribosomal protein genes, and some genes that are targeted to the mitochondrion, were examined. Mitochondrial genes appear to be more diverse in sequence than nuclear genes. Comparison of mitochondrial divergence within *Acanthamoeba* is also juxtaposed with that from sequences of the limited number of mitochondrial genomes from other FLA within the Amoebozoa, *Balamuthia* and *Vermamoeba*. In general, mitochondrial genes from *Acanthamoeba* were much more diverse than those of other FLA.

O1.2

Systematic analysis of *Acanthamoeba* sp. sequence type correlated with source and pathogenicity in Italy

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Keywords: Acanthamoeba, One-Health, Italy

Acanthamoeba have been frequently reported as a potentially pathogenic free-living protozoan, causing a sight-threatening keratitis (AK), fatal granulomatous encephalitis (GAE), and cutaneous disorders. Based on rRNA gene sequences (ASA.S1 fragment), the genus is divided into 23 genotypes (T1 – T23). Particularly, the genotype T4 has been the most associated with AK and GAE. By 2001, it had become evident that also many isolates in environmental studies were classified into sequence type T4. Clustering of AK cases or tracking of strains in the environment might be possible by examining subsequences (alleles) within the ASA.S1 fragment [1]. The aim of the study is to analyze the distribution of *Acanthamoeba'* clinical and environmental samples in Italy by phylogenetic and statistical analyses. Sequence data retrieved from GenBank were compared with new positive' *Acanthamoeba* clinical samples obtained in the present study.

Clinical samples: Ten samples were collected at the Laboratory of Parasitology of the Polyclinic Tor Vergata of Rome and *Acanthamoeba* specific PCR was used to identify the genotype.

For database' creation: we search all the deposited *Acanthamoeba* sequences from Italy in PubMed Nucleotide using a multistep strategy matching the keywords *Acanthamoeba*, Italy, Italian, FLA and free-living amoeba. A GenBank "complete record" check was performed (last update March 2022) paying attention to "Country Isolation and Source".

Genotypes and Alleles characterization: 18S sequences spanning the spectrum of Italian *Acanthamoeba* diversity were downloaded and aligned with those produced here. Alleles identification was based on the Fuerst database [1]. Phylogenetic and statistical analyses were performed using RStudio.

All new clinical samples characterized in the present study belonged to genotype T4. Our multistep strategy produced a unique database with >120 sequences trimmed down by removing all input characterized in different genetic loci. Genetic analysis comprising the spectrum of Italian *Acanthamoeba* confirmed the existence of different genotypes – T2, T3, T4, T11 and T15 – in our country. New allelic variations never reported so far, were identified particularly in the genotype T4 and T15. The phylogenetic analysis did not allow to solve the genetic relations among isolates from different sources.

O1.3

Geographic distribution and genetic diversity of pathogenic free-living amoebae in the northern region of Mexico

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Keywords: Naegleria; Acanthamoeba; genetic diversity.

Information on the presence and genetic diversity of free-living pathogenic amoebae of the genera *Naegleria* and *Acanthamoeba* in Mexico is still null or scarce in some states. For this reason, we decided to begin the search for these organisms in the northern region, taking into consideration the sampling season and natural water bodies. In late September and early October 2020, 2021, and 2022, 28 water samples were taken from seven northern states. Samples of one liter of river water, canals, lagoons, streams, dams, waterfalls, and oases were taken. In the laboratory, 50 ml was measured after shaking and concentrated by centrifugation. The pellet was plated in duplicate on NNE plates and incubated at 37 and 42°C for amoeba isolation. Before 24 hours of incubation, small clusters of amoebas that were separated and with different trophozoite morphology were selected. DNA will be extracted from each strain for amplification and pre-identification by PCR, using specific primers for *Acanthamoeba* and *Naegleria* and universal primers for other genera of amoebas. The final identification will be made by sequencing the PCR products. So far, around 299 pure strains of free-living amoebae have been obtained, identified by morphology using an inverted microscope. 172 strains were isolated at 37°C and 127 strains grew at 42°C. Preliminary morphological and molecular results reveal the free-living amoebae of the genera *Naegleria*, *Acanthamoeba*, *Vermamoeba*, *Willaertia*, and *Mayorella*, among others. Furthermore, the selection of the isolation temperatures, chosen to search for the pathogenic free-living amoebae described so far, influenced the genetic diversity of the amoebae found and reported for the first time in the sampled places.

O1.4

Naegleria genus pangenome reveals new structural and functional insights into the versatility of these free-living amoebae

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Keywords: Naegleria; whole genome sequencing; pangenome

Free-living amoebae of the Naegleria genus belong to the major protist clade Heterolobosea and are ubiquitously distributed in soil and freshwater habitats [1,2]. Of the 47 Naegleria species described, N. fowleri is the only one being pathogenic to humans, causing a rare but fulminant primary amoebic meningoencephalitis [3]. Some Naegleria genome sequences are publicly available [4–10], but the genetic basis for Naegleria diversity and ability to thrive in diverse environments (including human brain) remains unclear. Herein, we constructed a high-quality Naegleria genus pangenome to obtain a comprehensive catalog of genes encoded by these amoebae. For this, we first sequenced, assembled, and annotated six new Naegleria genomes. Genome architecture analyses revealed that Naegleria may use genome plasticity features such as ploidy/aneuploidy to modulate their behavior in different environments. When comparing 14 near-to-complete genome sequences, our results estimated the theoretical Naegleria pangenome as a closed genome, with 13,943 genes, including 3,563 core and 10,380 accessory genes. Comparative analyses highlighted a remarkable genomic heterogeneity, even for closely related strains and demonstrate that Naegleria harbors extensive genome variability, reflected in different metabolic repertoires. If Naegleria core genome was enriched in conserved genes essential for metabolic, regulatory and survival processes, the accessory genome revealed the presence of genes involved in stress response, macromolecule modifications, cell signaling and immune response. Commonly reported N. fowleri virulence-associated genes were present in both core and accessory genomes, suggesting that N. fowleri' s ability to infect human brain could be related to its unique species-specific genes (mostly of unknown function) and/or to differential gene expression. The construction of Naegleria first pangenome allowed us to move away from a single reference genome (that does not necessarily represent each species as a whole) and to identify essential and dispensable genes in Naegleria evolution, diversity and biology, paving the way for further genomic and post-genomic studies.

O1.5

Free living amoeba isolation from waters and soils samples in Tenerife, Canary Islands, Spain.

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Keywords: free living amoebae, environmental samples, Acanthamoeba

Free-living amoebae (FLA) are ubiquitous protozoa commonly found in water and soil environments. FLA belonging to various genera, including Acanthamoeba, Balamuthia, Naegleria, and Vermamoeba, can cause opportunistic infections in humans and animals such as keratitis or meningoencephalitis. In addition, some of them serve as hosts for a large number of pathogenic bacteria, yeasts, and viruses. The aim of this work was to evaluate the presence of free-living amoebas in soil and water samples collected between 2021 and 2022 in Tenerife Island, Spain. The water samples were subjected to the membrane filtration technique, while soils were grown directly on plates of 2% non-nutrient agar (NNA). Both samples were incubated at room temperature and monitored daily for the presence of free-living amoebae. DNA was performed from the plates on which there was an abundant growth of FLA. Finally, PCR amplification of the 18S rRNA gene and sequencing of the DF3 region of Acanthamoeba 18S rDNA was performed.

The analyzed samples were collected during the months of 2021 and 2022 in different points of Tenerife. Soil samples were obtained from school gardens and private farms, whereas water samples were collected from ornamental fountains located in parks and recreational areas, taps, fish tanks among others. FLA were detected in ANN in all soil samples, and mostly in water samples. The genus Acanthamoeba was the most isolated according to molecular analysis.

O1.6

Isolation and identification of pathogenic Acanthamoeba species over time across anthropogenically polluted riverine systems

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Keywords: Acanthamoeba, environmental, pathogenic

Acanthamoeba species are known to be ubiquitous within the environment and have been isolated from a wide range of environments and matrices e.g. waters, soils, sediment, dust and biofilms (1 – 5). Some species are known to act as opportunistic pathogens, as well as being known to harbour bacteria, either as endosymbionts or as prey, with studies suggesting that bacteria can develop amoebae-resistant genes (6, 7). Furthermore, there is growing evidence suggesting *Amoeba's* role in the transmission of bacterial diseases and antimicrobial resistance, by acting as a vector.

As there has been a link established between historical pollutants and resistance (8-11) it was deemed plausible that these pollutants could be having an impact on the environmental microbiome. Specifically, in this study we look at how different geochemical factors can influence *Acanthamoeba* species, with a hypothesis that hostile / stressful environments can result in elongated periods of encystation, which could exacerbate bacterial gene transfer, including resistance.

Through a comprehensive study along the River Clyde, Glasgow, Scotland, we isolated *Acanthamoeba* sp. from sediment samples at different depths to ascertain differences in historical matrices and how different pollutants and geochemical parameters could impact the species present.

O1.7

Pathogenic free-living amoebae from water and soil sources from Cape Verde

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Keywords: environmental samples, free-living amoebae, Cape Verde

Free-Living Amoebae (FLA) are widely distributed protozoa, which contain some groups considered as pathogenic microorganisms. These members are able to produce several opportunistic diseases including epithelial disorders, such as keratitis and fatal encephalitis. Acanthamoeba spp., Naegleria fowleri, Balamuthia mandrillaris, Sapinia pedata, Vahlkampfia spp., Paravahlkampfia spp. and Vermamoeba vermiformis have been reported not only as causal agents of several opportunistic diseases, but also as capable to favour the intracellular survival of common pathogenic bacteria, which could avoid the typical water disinfection systems, non-effective against FLAs cysts. Even though they have been reported in numerous sources, such as soils, dust and water, there is no legislation related to the presence of these protozoa in soil and water-related environments worldwide. Therefore, there are no established prevention or disinfection protocols to advise the population regarding FLA infections or eliminate these microorganisms from human-related environments to date. Thus, the aim of the present study was to evaluate the presence of potentially pathogenic FLA in human-related soil samples and water sources of Santiago Island, Cabo Verde. A total of 12 water sources samples were filtered and 26 soil samples were seeded in non-nutrient agar plates (2%), incubated at 26 ◦C, and monitored daily to evaluate the presence of FLA. DNA was extracted from those plates on which there was suspected FLA growth, and PCR amplification of the 18S rRNA gene was carried out. Presence of Acanthamoeba species, most of them belonging to the virulent T4 genotype, and V. vermiformis, enhances the importance to control the protozoa contamination in human-related water sources and soil samples. Finally, as S. dejonckheerei is a recently discovered species, we consider it important to notify its presence in human-related environments. To the best of our knowledge, this is the first report of FLA presence in Cape Verde and the first report of V. vermiformis in beach sand worldwide.

O1.8

Development of multi-locus genotyping tools for the "brain-eating" ameba *Naegleria fowleri*

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Keywords: Naegleria; genotyping; brain-eating ameba

Background: The "brain-eating" free-living ameba, *Naegleria fowleri*, causes primary amebic meningoencephalitis (PAM), a rare brain infection that is almost always fatal. The ameba is found globally, and genomic signals have been detected in warm fresh waters, hot springs, and improperly maintained recreational water venues. Recently, a fatal PAM case from the United States was linked to an improperly treated public drinking water system. It is unclear why PAM cases are rare despite the widespread prevalence of *N. fowleri*, particularly in untreated recreational freshwater. Variability in the ameba's virulence may explain this. However, a genotyping system capable of categorizing *N. fowleri* strains based on virulence does not exist. Our goal in this study was to develop a highly discriminatory genotyping tool for improved strain characterization.

Methods: Using the Illumina HiSeq platform, we performed whole genome sequencing of 53 *N. fowleri* strains isolated from PAM patients and the environment, representing all three known U.S. genotypes. We used 100 repetitive DNA sequences in the nuclear genome and evaluated their utility in strain typing using a panel of 15 *N. fowleri* strains isolated from PAM patients and the environment by PCR amplification and gel electrophoresis.

Results: We identified four loci that are *N. fowleri*-specific and showed promise in individual strain typing. Three of the four loci were used in genotyping of *N. fowleri* isolates (N=76) available at the CDC laboratory. A total of 19, 4, and 7 different amplicon patterns were detected based on the PCR product sizes in these three loci,
respectively. When the amplicon patterns of three loci were used, there is low probability that two unrelated strains of *N. fowleri* show matching patterns by chance. Amplicon sequencing to understand the basis of size differences is pending.

Conclusions: This multi-locus genotyping system improved differentiation and characterization of *N. fowleri* strains. The method has potential applications to categorize *N. fowleri* strains based on their virulence profiles, improve source tracking of clinical cases, and enhance molecular epidemiological surveillance.

Session II : cell biology of free-living amoebae

Chairpersons: Ascel SAMBA - Julia Walochnik

Keynote speaker: Katrina Velle (Amherst, MA, USA)

Defining the cell biology underlying pathogenic behaviors in a relative of the "brain-eating amoeba"

O2.1

Naegleria fowleri profilin based on RNA-seq. analysis

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Keywords: Naegleria fowleri; RNA-seq; profilin

Naegleria fowleri is a free-living amoebae that can cause a fetal central nervous system infection through the nasal cavity. Encystation is the formation of cystic form of an amoeba to protect itself from unfavorable conditions, in which stage-specific genes are expressed. In previous studies, we obtained the transcriptome profiles differentially expressed in *N. fowleri* cyst or trophozoites by RNA-Seq. Actin and its regulatory proteins play a key role in several essential cellular processes such as cell movement, intracellular trafficking and cytokinesis in most eukaryotes. Profilin is known as an actin-related protein involved in the dynamic turnover and reorganization of the actin. In this study, we cloned *profilin* gene and characterized its function associated with *nf-actin*. The *nf-profilin* gene is composed of 450 bp (encodes 150 amino acid) and produced 22.5 kDa recombinant protein (rNf-profilin). The sequence identity was 83 % with nonpathogenic *N. grueri*. Anti-Nf-profilin antibodies was produced in Balb/c mice immunized with rNf-profilin. In addition, the anti-Nf-profilin antibody reacted with *N. fowleri* lysates but did not bind to lysates of other reference amoebae in western blot analysis. Using immunofluorescence assay, the Nf-profilin was localized on the pseudopodia in *N. fowleri* trophozoites. In contrast, the nf-actin was localized on cytoplasm including pseudopodia and food-cup structure. These results suggest that actin/profilin plays an important role in *N. fowleri* infection and pathogenesis of amoeba-host interaction.

O2.2

High efficiency transfection of *Acanthamoeba castellanii* using a cationic polymer

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Keywords: Acanthamoeba, transfection

The free-living amoeba *Acanthamoeba castellanii* is an ecologically, clinically, and evolutionary important microorganism. *A. castellanii* amoebae are directly pathogenic to humans, and serve as reservoirs and training grounds for bacterial pathogens (e.g., *Legionella pneumophila*), but also regulate the proliferation of other microorganisms in the soil. Despite their importance, no reliable genetic system has been developed, hampering the use of *A. castellanii* and related species as model organisms. Transfecting *A. castellanii* is possible with commercial kits, but is expensive, inefficient, and vulnerable to product discontinuation. In this contribution, we present a method for efficient transfection of *A. castellanii* with readily available and inexpensive cationic polymers – polyethylenimines. We systematically explore the parameters of the method, obtaining up to 100-fold higher efficiency than currently used reagents. The method presented here provides a robust step towards a full genetic toolbox for *A. castellanii*, hence expanding its use as a model organism.

O2.3

Alternative splicing and intron retention analysis during encystment and programmed cell death in *Acanthamoeba*

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Keywords: alternative splicing, intron retention, Acanthamoeba

Acanthamoeba is a cosmopolitan amoeba capable of producing human disease such as *Acanthamoeba* keratitis and granulomatous amoebic encephalitis. *Acanthamoeba* is a protozoan with a biphasic life style that includes a vegetative trophozoite and a latent cyst. It also has the peculiarity of presenting programmed cell death given certain conditions. The expression of *Acanthamoeba* genes has been analyzed to study different processes using microarrays, qPCR and RNAseq. However very little has been researched in regards to alternative splicing events. Alternative splicing are common mechanisms by which different transcripts from the same gene can increase the number of potential proteins. One of the main forms of alternative splicing is intron retention. Recently, intron retention was shown to occur differentially in 67 genes during encystment (after 24 and 48 hours of inducing the process). Here we describe intron retention events during programmed cell death. We had 2 treatments with 3 samples each. One of the treatments consisted of trophozoites while the other one included cultures where programmed cell death was induced and RNA was obtained after an hour. We analysed the data obtained using iREAD and IRFinder and separated intron retention events where the overrepresentation was caused by gene overexpression. We found over 60 genes that presented retained introns during programmed cell death. Additionally, we describe opportunities that come from studying intron retention and other alterntaive splicing events in *Acanthamoeba* that are poorly understood. Studying alternative splicing in *Acanthamoeba* and similar organisms can provide a better understanding of their biology, and new options for treatment to combat the diseases produced by them.

O2.4

Molecular atlas of *Acanthamoeba castellanii* remodeling during cyst formation

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Acanthamoeba spp. are free-living amoebae found in soil, water, and air. They cause fatal infections in the human central nervous system (encephalitis) and are responsible for *Acanthamoeba* cornea keratitis, an eye infection that can result in blindness. They are also known to host pathogenic bacteria such as *Legionella* spp. and *Mycobacterium avium*, to name a few. *Acanthamoeba* spp. present a two-phase life cycle: (*i*) an active phase that feeds on bacteria and available wastes, (*ii*) a dormant cyst phase triggered by stress or starvation. The latter is characterized by a round shape and double-walled protection which makes the amoeba very resistant. It is thus important to understand mechanisms involved in cyst formation in the context of medical treatment.

Here, we mapped the molecular changes occurring in *Acanthamoeba castellanii* during cyst formation using high throughput transcriptomics, proteomics and phosphoproteomics. We identified 166,782 transcripts and 8,577 proteins that were monitored up to 8h after triggering cyst formation *in vitro*. RNAseq identified more than 100,000 previously undescribed transcripts that were used for protein identification. This strategy allowed the identification of 2,701 proteins absent from *A. castellanii* reference proteome. Overall, 3,270 proteins were quantified at transcript- and protein-level, constituting the first time-resolved molecular atlas of *A. castellanii* remodeling during cyst formation. We observed a delay between transcript- and protein-level regulation. 443 proteins presented significant variation at protein-level while nearly 500 were regulated by phosphorylation and/or dephosphorylation. Of these, only 11% were regulated at both protein- and phosphorylation-level. These results confirm the involvement of phospho-regulation in *Acanthamoeba* spp. cyst formation while providing relative quantification of 6,376 individual phosphorylation sites.

This work is the first multi-OMICs data set exploring amoeba encystment from transcripts to post-translational modifications. We annotated the genes using eggNOG-mapper to provide functional information and performed statistical analysis that identifies proteins and phospho-regulations potentially involved in cyst formation.

O2.5

Unravelling the biology of *Balamuthia mandrillaris* survival in a physiological relevant context

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To survive in the human body, the environment-dwelling amoeba must adapt to the extreme changes of the niche. *Balamuthia mandrillaris* trophozoites receive nutrients from the surrounding cells, likely contributing to fatal brain damage. Although it belongs to a phylum of Amoebozoa similar to *Entamoeba histolytica*; however, the mechanism underlying host cell uptake of trophozoites reportedly varies, likely independent of phagocytosis. Cellular ingestion of *B. mandrillaris* trophozoites was investigated in both traditional cell culture and three-dimensional systems. A clinical isolate of *B. mandrillaris* trophozoites was cocultured with human neuroblastoma SH-SY5Y cells, neurosperoid and cerebral organoid. Host-parasite interactions were observed in a three-dimensional (3D) manner using confocal and holotomographic microscopes with and without fluorescence labelling. In the 2D culture, *B. mandrillaris* trophozoites protrude cytoplasm into human neural cells, a structure mimicking invadopodia. Live imaging reveals human protein internalisation in different patterns, including membrane-bound granules, nonmembranous granules and cytoplasmic dispersion. The intervention of trogocytosis could not inhibit this host cell uptake. Moreover, using the 3D cell culture show decrease in human cell survival, similar to the pathological observation in the human brain. Taken together, *B. mandrillaris* trophozoites ingest human cell components independent of trogocytosis. The 2D and 3D coculture of the trophozoites yielded different behaviour. Elucidation of pathogen-specific survivability may pave the way to reducing brain damage and more specific therapy.

O2.6

Genotype-dependent motility and encystment within genus *Acanthamoeba*

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Keywords: Acanthamoeba, motility, encystment

The genus *Acanthamoeba* are free-living amoeba with two cell forms, an active motile trophozoite and a dormant cyst. *Acanthamoeba* are largely characterized by genotype using the 18S Ribosomal RNA gene. The most studied genotype is T4 due to its association with human infection. The *Acanthamoeba* genus continues to diversify as more genotypes are identified. *Acanthamoeba* is specifically interesting due to its ability to encyst due to nutrient unavailability and its ability to forage for nutrients when motile, generally for bacteria. We hypothesized that different genotypes would demonstrate different motility and encystment potential over time.

To determine *Acanthamoeba* motility, *Acanthamoeba* strains from genotypes T1, T2, T3, T4, T5, T7, T11 and T18 were examined. *Acanthamoeba* trophozoites were plated in 48-well plates and time-lapse videos were collected for up to 72 hours, in the absence or presence of *E. coli*. Individual trophozoites were tracked using TrackMate in ImageJ to determine the max speed and total distance for each genotype. Concurrently, the amount of encystment over the course of the experiment was collected by enumerating calcofluor white-positive cysts versus total cell count (trophozoites + cysts).

Acanthamoeba from the T5 genotype had a significantly higher total distance compared to all other genotypes in the first hour of motility ($p<0.05$). At 24 hours, both T4 and T5 had significantly higher total distance compared to all other genotypes ($p<0.05$). PRA-115 had the lowest total distance traveled at 24 hours compared to all other genotypes which reflects the high amount of encystment seen for this strain. At 24 hours, nearly 100% of PRA-115 cells had encysted. No other strain demonstrated such rapid encystment in such a short timeframe in the absence of nutrients. The average max speed was observed in T1 and T4 at 1.25 micron/sec.

While the *Acanthamoeba* genus is diverse, motility and encystment remain common biological features across the genotypes. Here, we demonstrate that genotypic differences in motility and encystment. The next step would be to evaluate the differences in motility and encystment against the potential to infect humans as both behaviors have significant implication for human disease.

O2.7

Multi-omics analysis of *Willaertia magna* C2c Maky: pathway to the discovery of biologically active compounds

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Keywords: free-living amoeba; metabolomics; Willaertia magna

Willaertia magna C2c Maky is a non-pathogenic free-living amoeba isolated from thermal waters, capable of phagocytosis and controlling microbial populations including Amoeba-Resisting Bacteria (ARB) [Hasni *et al.,* 2020; Croze *et al.,* 2021] or some fungal species (unpublished data). In addition, the lysate of this amoeba displays antifungal properties against various phytopathogens [Demanèche *et al*., 2020; Troussieux *et al.,* 2022].

The analysis of the genome, transcriptome and proteome of *W. magna* C2c Maky revealed genes involved in antimicrobial activity [Hasni *et al.,* 2019; Hasni *et al.,* 2020]. Metabolomic approach requires to control the set of culture parameters in order to notice subtle variations. One obstacle is the residual culture medium in the samples which masks the amoebic compounds. The biotech company AMOEBA, has successfully developed a medium for the axenic culture of *W. magna* C2c Maky in large-scale bioreactors as well as a procedure to remove the media, thus providing optimal conditions for metabolomic analysis.

Objectives: To date, very limited knowledge is available on the nature, concentration or function of amoebic compounds. To fill this gap, we **(i)** assessed the influence of culture conditions on the production of amoebic metabolites and **(ii)** compared the metabolomic profiles of three closely related strains [Hasni *et al.,* 2020] of *W. magna* to determine the specificity of the ARB-resistant strain C2c Maky.

Method: We cultivated axenically in cell factories the four described strains of *W. magna*. When confluent, the amoebae were centrifuged and then rinsed to get rid of the culture medium. The molecules were extracted by liquid-liquid partitioning and analyzed comparatively by UHPLC-ESI-QTOF and GC-MS.

Results: We detected several compounds specific to the C2c Maky strain. We have also highlighted families of molecules affected by the bioreactor culture conditions, in particular apolar compounds.

O2.8

Presence of the 19S subunit of the proteasome in trophozoites of *Naegleria* genus

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Keywords: Proteasome, Naegleria sp. Regulatory particle

Naegleria genus is part to the group of free-living amoebae. Within this genus are *N. fowleri,* the only pathogenic species for humans responsible for primary amoebic meningoencephalitis (PAM), a highly fatal disease of the central nervous system and the non-pathogenic species *N. gruberi* used as a cell differentiation study model and *N. lovaniensis* the closest relative of *N. fowleri* but without the capacity to produce the disease in humans or animals. On the other hand, the 26S proteasome is a multisubunit protease and is the most important catalytic complex for recycling and degradation of intracellular proteins in eukaryotic cells. This machinery is composed by the catalytic core (20S) that is associated with degradation function, and the regulatory particle (19S). The 19S recognizes and processes ubiquitinated proteins, leading them to degradation. In many genera of pathogenic protozoa, this complex has been characterized and involved in various biological processes (proliferation, differentiation, etc.); however, in *Naegleria* genus only the 20S subunit is known. The aim of this project is to demonstrate the presence of 19S subunits in *N. fowleri*, *N. gruberi* and *N. lovaniensis.* Through experimental trials such as western blot and immunolabeling, we stlablished the presence and localization of the 19S subunit. Our acknowledgement to CONACYT for financial support (CVU: 997938) provided during the project.

O2.9

Enzyme treatments promoting trophozoite egress from *Acanthamoeba castellanii* cysts

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Keywords: Acanthamoeba, excystment, enzymatic digestion

Acanthamoeba sp. is a Free-Living Aoeba (FLA), which can be found in many natural and artificial environments. This FLA can cause serious ocular (amoebic keratitis) or cerebral (granulomatous amoebic encephalitis) infections. During its life cycle, depending on environmental conditions, *Acanthamoeba* alternates between a vegetative and mobile form, called the trophozoite, and a resistant form with low metabolic activity, called the cyst. The cyst wall of Acanthamoeba sp. is mainly composed of carbohydrates and proteins, with an inner and an outer layer, called the endocyst and the ectocyst, respectively.

The aim of our study is to stimulate *Acanthamoeba castellanii* excystment by managed enzymatic digestion of the main components of its cyst wall, by using proteases (pepsin or collagenase) or glycosidases (chitinase or cellulase). After a treatment of *Acanthamoeba* cysts with these enzymes, the proportion of cysts and excysted trophozoites was determined in microscopy. Cyst walls were further analyzed for their carbohydrate distribution in fluorescence microscopy and at the ultrastructural level in transmission electron microscopy.

After treatment by cellulase, collagenase and pepsin, a total excystment of *A. castellanii* was observed after only 2 days, while 11 days were necessary for the untreated control, showing a considerable acceleration of trophozoite egress from cysts following treatment by these three enzymes. No effect was observed on *A. castellanii* excystment after chitinase treatment. The analysis of cysts labeled by lectins conjugated with fluorescein showed a tendancy of decrease of endocyst labeling in cellulase-treated amoebae compared to untreated control. In electron microscopy, cellulase-treated cysts displayed a significant decrease of their intercystic space.

Our data show that managed enzymatic digestion by cellulase, pepsin and collagenase lead to cyst wall ultrastructural modifications and to an excystment stimulation of *A. castellanii*.

O2.10

Six new insights from a close examination of the abundant cyst wall proteins of *Acanthamoeba*

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Keywords: cyst wall, lectins, enzymes

The cyst wall of *Acanthamoeba castellanii*, the free-living amoeba that causes keratitis, contains cellulose, chitin, and (possibly) xylan and has ectocyst and endocyst layers connected by conical ostioles. Previously, we used mass spectrometry of purified walls to identify three families of abundant lectins (Jonah, Luke, and Leo), as well as an abundant laccase. Here we present six new insights into these proteins based upon a close examination of their expression, localization, and structure. First, strand-specific RNA-Seq and whole cell proteomics showed all of the lectins are encystation-specific, but some are expressed early, while others are expressed later, with substantial mixing within some families. Second, confocal microscopy of GFP-tagged proteins on their own or another promoter show that proteins expressed early localize to the ectocyst layer, which is made first, while proteins expressed late localize to the endocyst layer and ostioles, which are made second. Third, because Jonah and laccase localize to distinct places in the ectocyst layer, and Luke and Leo localize to distinct places in the endocyst layer and ostioles, pairs of proteins are binding to different, as yet unspecified, glycopolymers. Fourth, AlphaFold and Foldseek show 3 of 4 cyst wall proteins share common ancestry and have structures similar to those of bacteria (one or three β-helical folds of Jonah, two or three β-sandwiches of Luke, and three copper oxidase domains of the laccase). These proteins derive from bacteria by lateral gene transfer, but a precise donor cannot easily be identified. In contrast, two sets of disulfide knots of Leo lectins are unique. Fifth, despite their unrelated structures, Luke and Leo lectins each contain linear arrays of aromatic amino acids (Trp, Phe, and Tyr), which are necessary for proper localization in the cyst wall. Similarly, a carbohydrate-binding domain in an *Acanthamoeba* endocellulase contains a linear array of aromatics necessary for binding cellulose and for proper localization in the cyst wall. These are examples of convergent evolution. Sixth, during excystation, the endocyst layer marked by Luke is broken down first, while the ectocyst layer marked by Jonah is broken down second. The sequence of how the wall is broken is opposite from how it is made.

Session III: interactions of free-living amoebae with other microorganisms

Chairpersons : Yann Héchard - Maritza Omana

Keynote speaker: Thierry Soldati (Geneva, Switzerland)

Cell-autonomous Mechanisms of Sensing and Defence against Mycobacteria Infection in Dictyostelium

O3.1

Abortive infection and defensive symbiosis – how amoebae defend against giant viruses

Authors: *Patrick Arthofer, Vincent Delafont, Alban Hay, Anouk Willemsen, Florian Panhölzl, Matthias Horn*

Free living amoebae, like all cellular organisms, frequently have to cope with viral infections. The main group of amoeba infecting viruses, the giant viruses, changed our perception of the viral world. With genome and particle sizes comparable to those of bacteria and a number of cellular features, giant viruses sparked a controversy about their evolutionary origin. Frequent host-switches, and competition with other viruses and bacterial symbionts were proposed as drivers of giant virus evolution. While virophages have been shown to facilitate protist host survival during giant virus infection, very little is known about other defense mechanisms. Here, we report on two novel defense strategies observed with two different giant viruses in two very dissimilar amoebae hosts. First, we studied the role of bacterial symbionts of free-living amoebae in the establishment of giant virus infections. To investigate these interactions in a system that would be relevant in nature, we isolated a giant virus (Marseilleviridae) and an *Acanthamoeba* host infected with a bacterial symbiont, identified as *Parachlamydia acanthamoebae*, from the same environmental sample. Systematic co-infection experiments showed that the bacterial symbiont represses the replication of the sympatric giant virus as well as other giant viruses (Mimiviridae) in both environmental isolates as well as *Acanthamoeba* lab strains. Second, we studied a new giant virus isolate (Mimiviridae) infecting members of the amoeboflagellate genus *Naegleria*. We describe how infection of *Naegleria* may lead to abortive infection, in which viral replication and dissemination is blocked by premature host cell death, ensuring the survival of the amoeba host population. Together, we show that amoebae employ diverse and as yet underexplored strategies to cope with omnipresent giant viruses.

O3.2

Interactions between free living amoebae and Cryptosporidium parvum in both planktonic and biofilm conditions.

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From 2004 to 2016, Cryptosporidium was responsible for 905 (60%) of worldwide reported waterborne outbreaks caused by protozoan parasites. Free Living Amoebae (FLA) and Cryptosporidium oocysts occasionally share the same environment. The aim of this study was to evaluate interactions between oocysts of C. parvum and two common FLA (Acanthamoeba castellanii and Vermamoeba vermiformis) in water environment. Interactions were studied in both planktonic and biofilm conditions over time. Encystment and survival of FLA were evaluated by microscopy using trypan blue vital coloration. Oocysts were enumerated on microscopy. Potential phagocytosis was evaluated by several microscopic approaches. Oocysts infectivity was evaluated by cell culture associated with quantitative PCR. Occasional phagocytosis of C. parvum by FLA was documented. However, oocysts concentrations did not decrease significantly. Due to physical interactions, the infectivity of oocysts was transitory decreased in presence of A. castellanii. Additionally, the biofilm condition could favor the persistence or even the proliferation of oocysts over time. This study clearly demonstrated interactions between C. parvum and FLA depending on conditions. Influence on C. parvum infectivity is specifically interesting. Further knowledge of mechanisms implicated in decrease of oocysts infectivity in presence of A. castellanii will potentially be of great interest, especially for therapeutical approaches.

O3.3

Environmental free-living amoeba as potential reservoirs for Mycobacterium bovis and Mycobacterium avium subsp. paratuberculosis

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Mycobacterium bovis (Mbo) and *Mycobacterium avium* subsp. *paratuberculosis* (Map) are two bacteria responsible for bovine diseases, tuberculosis and paratuberculosis respectively. Depending on the species, they can be digested, survive or grow within FLA. Studies have shown that *Mycobacterium bovis* (Mbo) and *Mycobacterium avium* subsp. *paratuberculosis* (Map) can grow in FLA in vitro but few studies reported this interaction in an environmental context. Understanding mycobacterial survival and persistence in the environment is important to better understand their life cycle. Our hypothesis is that FLA can be an environmental reservoir and vector of Mbo and Map.

To test this hypothesis, we concentrate our work on farm positive for Mbo or Map in France to isolate and characterise environmental FLA associated with Mbo or Map. Water and soil samples from infected farms were collected to isolate FLA. Total DNA from these FLA was used to detect Mbo and Map DNA using a nested qPCR. Subsequently, the environmental FLA were used to characterize their interaction with Map by infection assays.

The nested qPCR allowed us to detect some Mbo and Map DNA suggesting a putative association within the environment. Different environmental FLA were isolated and characterised. Three of them were chosen to investigate the permissiveness by infection assays using a Map-GFP strain. Multiplication of infected FLA was explored by videomicroscopy suggesting that Map-GFP could be transmitted vertically during FLA division. Our work led to isolate many environmental FLA from infected farms, to detect the presence of Mbo and Map DNA in environmental FLA, and to show the permissiveness of three different environmental FLA to Map infection. These results highlight the possible role of FLA as reservoirs of Mbo and Map in the environment.

O3.4

Exploring the interactions of free-living pathogenic amoebae with bacteria and host cells.

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Keywords: Naegleria fowleri, Acanthamoeba castellanii, virulence

Worldwide distributed free-living amoebae *Naegleria fowleri* and *Acanthamoeba castellanii* are found in soil and aquatic environments where they feed on bacteria and other protists. On rare occasions, these organisms can cause deadly diseases in humans with high mortality. In this study, our aim is to describe the interactions of both amoebae with bacterial and mammalian cells at the protein level.

To quantify the virulence of amoebae, we developed a method in which the feeding on host cells is monitored using flow cytometry with fluorescently labelled cells. To observe the short-term effect of the presence of bacteria and mammalian cells, we incubated *N. fowleri* and *A. castellanii* with *Enterobacter aerogenes* or fibrosarcoma cells for 6 hours. In addition, we cultivated amoebae for several passages with mammalian cells and isolated *N. fowleri* from the brain of infected mice. All samples were subjected to comparative label-free proteomic analysis with axenic amoebae used as a control.

The two studied amoebae reacted differently to the presence of bacteria and host cells. *A. castellanii* generally showed significant proteome changes when co-cultured with both bacteria and host cells. The most pronounced changes were observed in proteins involved in cytoskeleton dynamics. In contrast, significant changes in the *N. fowleri* proteome were observed only upon long-term contact with the host. In this case, however, there were significant changes in various metabolic and functional pathways, and we were able to identify several known and novel virulence factors. We have chosen to characterize several of these and will discuss their possible function in the interactions with the host.

O3.5

Bacterial microbiome management in free-living amoebae isolated from water: the impact of amoebae identity, available food source and replication cycles.

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Keywords: Free-living amoebae; natural bacteriome; water ecosystems

Free-living amoebae (FLA) are ubiquitous protists found is soil and freshwater, mainly feeding on bacteria [1,2]. They are also well-known reservoirs and vectors for the transmission of amoeba-resistant bacteria (ARB), most of which are pathogenic to humans [3,4]. Yet, the natural microbiome of wild amoebae remains largely unknown [5,6] and the effects of amoeba identity and environmental factors on FLA bacterial microbiome composition unexplored. Monocultures of Naegleria australiensis, Naegleria KDN1, Paravahlkampfia ustiana and Vermamoeba vermiformis isolated from recreational geothermal waters in Guadeloupe were passaged with or without different food sources (E. coli, yeast, fetal calf serum and water) during successive replication cycles. The whole bacterial microbiome of the recreational baths and the amoebae was characterized using 16S rDNA metabarcoding. The culturable subset of amoebae-associated bacteria was analyzed by mass spectrometry (MALDI-TOF MS) and disk diffusion method to assess bacterial antibiotic resistance. Transmission electron microscopy allowed to locate the bacteria inside the amoebae cysts. Alpha and beta-diversity analyses revealed that amoebae microbiomes were different from the microbiomes of their water habitat. While Vogesella was the most abundant genus detected in the waters, the most common amoebae-associated bacteria belonged to Pseudomonas, Bosea, and Escherichia genera. These newly isolated FLA with distinct identity also showed both temporary and permanent associations with differentially abundant bacterial taxa, suggesting host specificity. These symbiotic relationships depended on the food source available and the number of replication cycles. Cysts were shown to carry viable bacteria of the Acinetobacter, Escherichia, Enterobacter and Pseudomonas genera, all being pathogenic to humans. Our results show that distinct amoebae can differentially select for traits that allow bacteria to be eaten or to become endocytobiont, depending on environmental conditions. The remanent presence of pathogenic bacteria inside resistant cysts through several replication cycles represents a potential health risk. It is thus imperious to improve knowledge on amoebae-bacteria interactions to ensure the continued safety of water systems and to further understand the evolution of bacterial endosymbiosis in these single-celled eukaryotic organisms.

O3.6

Free-Living Amoebae Diversity in Drinking Water and Investigating the Role of Preferential Grazing

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Keywords: Drinking water, Acanthamoeba, grazing

Free-living amoebae (FLA) are increasingly recognized as important predators in diverse ecosystems, including drinking water. However, FLA diversity and the role of FLA in drinking water systems are still being explored. This study sought to investigate 1) the diversity of FLA in a full-scale drinking water system, 2) the genetic variation within 18S rRNA genes of drinking water FLA isolates, and 3) the influence of preferential grazing on drinking water bacterial communities. Samples were collected over the course of one year, source-to-tap, in a full-scale chloraminated municipal drinking water system. A total of 21 isolates were recovered from 65 samples, including the genera Acanthamoeba, Vermamoeba, Naegleria, Vannella, Echinamoeba, and Rosculus. Acanthamoeba spp. were most frequently recovered, being isolated across seasons and throughout the treatment and distribution systems. The isolates were sent for PacBio long-read sequencing targeting a 4,500-basepair region of the small and large subunit RNA genes, which will facilitate species-level identification and analysis of variation in RNA sequences within single populations of diverse drinking water FLA species. Twenty water samples from the treatment plant filter effluent, finished water, and three distribution system sites were analyzed using metagenomics, revealing seasonal trends in different classes within the Amoebozoa supergroup. Preferential grazing by FLA is hypothesized to contribute to enrichment of opportunistic pathogens in drinking water systems, as many OPs possess anti-predation strategies, such as evading uptake via phagocytosis or interrupting digestion. Certain OPs, such as Legionella pneumophila, can infect FLA and replicate within them, causing cell lysis. To investigate the effect of preferential grazing by FLA on bacterial populations in the environment, the model FLA Acanthamoeba castellanii strain Neff (ATCC 30010) will be exposed to equal concentrations of Escherichia coli K-12, which possesses no known defenses against FLA, and three Ops with varying physiologies and anti-predation strategies (L. pneumophila, Pseudomonas aeruginosa, and Mycobacterium smegmatis). Concentrations of bacterial cells will be quantified over time using fluorescence microscopy and quantitative polymerase chain reaction (qPCR). Results are expected to show that A. castellanii will preferentially consume E. coli to avoid grazing on OPs, which could lead to the enrichment of OPs in the environment.

O3.7

Ecology of free-living amoebae and varying predation capacity against aquaculture related pathogenic vibrios in Mediterranean costal environments.

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Keywords*: Free-living amoeba diversity, Vibrio amoeba interactions, Marine microbial ecology*

Free-living amoeba (FLA) diversity, population dynamics and niche specialization in marine environment remain poorly described[1]. In a previous study, we observed a low diversity of amoebae in oyster farming area in the Thau lagoon in the south of France, with mostly Vannellid amoebae[2]. Herein, we wondered whether this low diversity is particular to this oyster farming environment and whether the presence of pathogenic vibrios potentially resistant to grazing can impact FLA communities. To address these questions, we sampled FLA monthly over an entire year along the Mediterranean coast, in three distant and contrasted sites, using various nutritive sources to follow FLA dynamics and predation capacity. By performing 18S barcoding, as well as clonal isolation, we found a diversity of amoebae belonging to *Vannellidae*, *Tubulinea*, *Rhizamoeba*, *Vermistella*, *Flabellulidae* and *Paramoebidae* families, and isolated a total of 301 clones representing this diversity. Our results reveal that beyond seasonal variations the overall FLA diversity found in the sediments is higher and different from the diversity found in the water column. Moreover, FLA diversity found in the sediment is specific for each sampling sites on the contrary to the FLA diversity found in the water column. Finally, we observed different predation capacity of phylogenetically related groups of FLA against different vibrios, that are bivalve opportunistic pathogens, suggesting complex predator-prey interactions that could play a role in pathogen dynamics in these environments.

O3.8

Role of oxidative stress in *A. castellanii* infection by *L. pneumophila*

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Keywords: Legionella pneumophila, free-living amoebae, oxidative stress

Legionella pneumophila is a facultative intracellular bacterium that proliferates in hot water notably in man-made water supply systems. It causes legionellosis, a notifiable respiratory infection that affects about 1500 people per year in France and kills about a hundred. Transmission to humans occurs after inhalation of contaminated water droplets, via showers or air conditioning systems for example[1]. *L. pneumophila* reaches the alveolar mucosa and multiplies inside macrophages thanks to its ability to resist phagocytosis. In water, *L. pneumophila* develops mainly inside amoebae such as *Acanthamoeba castellanii*, which fed on bacteria by phagocytosis. *L. pneumophila* has developed strategies to resist this phagocytosis, to exploit the nutritional inputs of the host, and to intercept its metabolites[2]. We know that *L. pneumophila* infecting *A. castellanii* impacts amoebae proliferative abilities and reduces its velocity[3,4], but little is known regarding the impact of the infection on *A. castellanii* metabolism. Using a proteomic approach, we have shown that infection of *A. castellanii* by *L. pneumophila* leads to a modification of the expression of proteins involved in host's metabolic pathways in a type IV secretion system (T4SS)-dependent manner. Our results suggest the involvement of defense systems related to oxidative stress, which have been poorly described in amoebae. A fluorescent labeling approach allowed us to show that *L. pneumophila* can decrease the amount of ROS inside *A. castellanii* likely through their T4SS. Potential enzymatic targets related to the antioxidant defenses of the host are currently being analyzed by RT-qPCR. Finally, a targeted metabolomics analysis is assessing the impact of *L. pneumophila* infection on *A. castellanii* metabolites related to antioxidant defenses. The identification of new cellular targets in amoebae necessary for bacterial infection would pave the way for the development of new approaches to reduce *L. pneumophila* occurrence in water.

O3.9

Antimicrobial resistance (AMR) and Free-Living Amoebae – the overlooked vector?

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Keywords: Antimicrobial resistance, Free-living amoebae, Amoebae-resistant bacteria

Free-living amoebae (FLAs) are ubiquitous, cosmopolitan and unicellular protozoa that can be infected with bacteria that survive intracellular digestion, known as Amoeba-resisting bacteria (ARB) (Khan & Siddiqui, 2014; Shi et al., 2021). FLAs can then act as reservoirs, vectors and training grounds for potentially pathogenic microorganisms (Scheid, 2014). Furthermore, the accumulation of antimicrobial substances in the environment has a key role in the selection of the resistant microorganisms (Martins & Rabinowitz, 2020). This study investigated the role of FLAs on levels of AMR in heavily polluted environments. Sediment samples were collected up and downstream of a chemical wastewater treatment plant (WWTP), which predominantly processes pharmaceutical waste. The FLAs were isolated and characterised, then, were brought to the state of monocultures and mechanically opened to retrieve the intracellular bacteria. The isolated bacteria were screened for their AMR against seven classes of antibiotics and compared to the antimicrobial resistance pattern of the environmental bacteria selected from the corresponding sample. Results showed that among the FLAs, the *Acanthamoeba* species were predominant, with recurrent amoebic intracellular bacterial species: *Pseudomonas spp*. Furthermore, preliminary results suggest higher resistance within the intracellular bacteria in contrast to their environmental counterpart. These outcomes highlight the potential role amoebae have in AMR prevalence and will increase our understanding of protists' role in AMR.

O3.10

Saccamoeba sp. (Amoebozoa, Tubulinea) with double rozellid infection

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Keywords: *Nucleophaga*; *Morellospora*; *Saccamoeba*

The Rozellomycota are intracellular parasites of algae and other protists, placed towards the base of the fungal kingdom. Among the few known taxa are parasites of various free-living amoebae, residing in the cytoplasm (*Morellospora*) or nucleus (*Nucleophaga*, *Paramicrosporidium*) of the infected host and closely resembling primitive Microsporidia (1-3). However, they form separate lineages, the closest to Microsporidia currently being *Nucleophaga*. During our investigations, an amoeba showing possible signs of infection was recovered. The strain was maintained by serial passages on agar plates with bacteria as food, and rDNA analyses were performed. The morphological identification of the amoeba as belonging to the genus *Saccamoeba* (Amoebozoa, Tubulinea, Euamoebida) was confirmed by the sequencing of its 18S rDNA which proved to be clearly distinct from those of the other available species. In contrast, to identify the parasite, repeated testing of different subsamples yielded two distinct types of DNA fragments, corresponding to either *Morellospora* or *Nucleophaga*. Both fragments differed from the sequences of our other strains, excluding the possibility of contamination, indicating instead that they could correspond to new strains or species. Therefore, the recovered amoeba would exhibit dual infection with intracytoplasmic (*Morellospora*) and endonuclear (*Nucleophaga*) parasites. On visual inspection, however, the amoebae divided regularly, showing no nuclear enlargement as usually induced by *Nucleophaga* growth (4), and the only visible growing parasites were in the cytoplasm, therefore identifiable as *Morellospora*. It remains to be determined whether *Nucleophaga* is actually only present in a subset of the amoeba population and/or whether its growth is prevented by the host or inhibited by the presence of *Morellospora*.

O3.11

Paramoeba atlantica as a reservoir for *Vibrio bathopelagicus* a potential pathogen against marine bivalve

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Keywords: Paramoeba ; vacuole colonization , parasitism

Amoeba-bacteria interactions enclose a wide range of biological associations, from predator-prey relationships to mutualist symbiosis[1]. While bacteria of the Vibrio genus are ubiquitous in marine environments, and can interact with a diversity of organisms, the occurrence of their interactions with marine amoebae remains poorly characterized. Nevertheless, several studies have highlighted the potential role of these interactions in the environmental persistence and virulence acquisition by vibrios against humans and marine animals [2,3]. Here, we report a durable association between a newly identified strain of *Vibrio bathopelagicus* namely Pa22 and a marine amoeba, *Paramoeba atlantica*, isolated at 270 meters depth in the Atlantic Ocean. By studying the cell biology of this interaction, we observed that the *Vibrio bathopelagicus* Pa22 can colonize a vacuole inside the amoeba, in which they remain viable and highly motile. Additionally, the strain Pa22 shows strong virulence potential against oysters, and belongs to the Splendidus clade that contains members associated with bivalves' mortalities. Therefore, benthic amoebae of the *Paramoeba* genus could host potentially virulent bacteria against marine animals.

O3.12

Genomic insights into non-canonical Legionellales symbionts of Free-living Amoebae

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Keywords: Paramoeba ; Legionellales ; Bacterial comparative genomics

The Legionellales order is notoriously known for comprising human pathogens such as *Legionella pneumophila* and *Coxiella burnetti*, etiological agents of Legionnaire's disease and Q fever, respectively. Apart from those emblematic representatives, our knowledge on this taxonomic group dramatically expanded after understanding that numerous species and genera thrive by forming symbiotic associations with a variety of eukaryotic hosts, ranging from unicellular protists to isopods and arthropods. Added to this, recent sequences-based surveys estimated that Legionellales is much more diverse than previously thought, encompassing at least 450 uncultured genera[1]. In this line, recent research enabled the recovery of Legionellales endosymbionts in various free-living amoebae (FLA), forming novel clades within this order [2–4].

In this context, we aimed to investigate associations between free-living amoebae and Legionellales, in order to gain new genomics insights into those elusive intracellular bacteria.

Through various sampling campaigns, FLA isolates were screened for the presence of intracellular bacteria, using fluorescence in situ hybridization and electron microscopy. Among the tested isolates, two novel Legionellales-infected protists strains were identified, corresponding to two marine isolates affiliated to *Paramoeba atlantica.* Those were added to a previously described testate FLA isolated from a cooling water tower, affiliated to *Cochliopodium minus* and known to bear Legionellales. Endosymbionts were purified and extracted from those 3 FLA strains, and genome sequences were reconstructed, using high throughput Illumina Miseq sequencing. Global signature of a strict endosymbiotic lifestyle such as genome size reduction, AT bias, among others, were witnessed in all three genomes. A comparative genomic approach allowed to pinpoint shared and specific genomic features among those Legionellales representatives. It also provided a comprehensive framework for phylogenomics reconstruction of Legionellales, placing the three novel endosymbionts of FLA into two deeply branching clades, somehow related to Legionellaceae (endosymbionts of *Paramoeba*) and Coxiellaceae (endosymbiont of *Cochliopodium*).

In conclusion, genomic analyses of three new endosymbionts of FLA expanded once more the range of known intracellular bacteria tightly associated with FLA, as well as the knowledge on the Legionellales order of intracellular bacteria.

Session IV: infection and virulence of free-living amoebae

Chairpersons: Fernando Lares Villa - Fiona Henrichez

O4.1

Assessing Acanthamoeba-host cytotoxicity: comparison of common cell viability assays

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Keywords: Acanthamoeba-host cell interaction, viability, cytotoxicity

Acanthamoebae are free-living protists widely distributed in the environment worldwide and are known to be facultatively pathogenic for humans. They can cause, in healthy individuals, *Acanthamoeba* keratitis, a severe sight-threatening eye disease affecting mainly contact lens wearers. In immunocompromised individuals, they are causal agents of granulomatous amebic encephalitis, a fatal disease of the central nervous system. Although these infections are rare, they represent a big challenge for healthcare providers, since they have remained difficult to treat. Moreover, despite recent advances, their pathomechanism is still today not fully understood. This, however, may represent a keystone to improve existing and develop novel treatment approaches, and in addition, to get an insight into why some *Acanthamoeba* strains cause disease, while others do not.

In the present study, we investigated the cytotoxic effect of *Acanthamoeba* spp. on mammalian cells, in general, and on human corneal epithelial cells (HCEC), in particular, during their co-culture, by using and comparing common viability assays and correlating these results with microscopic observation. We could determine the most suitable and effective *in vitro* methods to detect and monitor the cytotoxicity effect induced by *Acanthamoeba* on the host cells.

Supported by the FWF (Project P 35259)

O4.2

Coordination of the in vivo immune response to *N. fowleri* infection

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Keywords: In vivo immune response; Naegleria fowleri; host:pathogen interactions

Naegleria fowleri, or the "brain-eating amoeba," is a free-living amoeba found in warm fresh water throughout the world. N. fowleri contact with the upper airway can cause a highly lethal infection known as primary amoebic meningoencephalitis (PAM), for which there is no effective therapy. Upper airway and brain invasion by N. fowleri provokes an intense immune response, but this response ultimately fails to control the parasite and contributes to severe immunopathology (1, 2). In vitro studies have suggested anti-amoebic roles for many host immune mechanisms (3-8), but how these in vitro findings relate to in vivo disease pathogenesis is unclear. In this study we sought to define immune mechanisms that are critical -or dispensable- for in vivo anti-amoebic immune activity. Identifying the factors underlying anti-Naegleria activity, will inform why these immune responses ultimately fail to control N. fowleri and provide opportunities to shape beneficial clinical responses. Using mice with germline or cell type specific gene deletions together with flow cytometry, static and live imaging techniques, and survival experiments, we have provided the first in vivo evidence for several non-redundant innate immune roles in coordinating amoebic pressure. We have also identified pathways and cell types that are surprisingly not necessary for the in vivo immune response to N. fowleri. Additionally, our lab has generated monoclonal antibodies that bind the amoebic cell surface and not only interfere with amoeba growth, but can target amoeba for immune mediated destruction. Antibody isotypes contain unique fragment crystallizable (Fc) regions with distinct effector functions (i.e. activating complement, engaging different Fc-receptors). By generating antibody isotype variants with we are able to specifically target protective innate effector functions to the amoeba. Intraventricular infusion of these anti-Naegleria antibodies into the cerebrospinal fluid significantly prolongs animal survival even when administered therapeutically. Our ultimate goal is to use our understanding of the in vivo immune response to N. fowleri to balance protective and pathologic immune activity to improve clinical outcomes for PAM.

O4.3

Characterization of secreted small RNAs of Naegleria fowleri and their potential as diagnostic biomarkers

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Keywords: Naegleria fowleri, smallRNAs, diagnostic biomarkers

The pathogenic free-living amoeba, Naegleria fowleri, is the etiological agent for primary amoebic meningoencephalitis (PAM), an acute brain disease with a case mortality rate of >97%. Several factors contribute to this considerable degree of mortality including delayed diagnosis, ineffective therapeutics and lack of understanding of the amoebic pathogenesis. To combat the notion of delayed diagnoses, we speculated that secretions of Nf could be harnessed as biomarkers to indicate infection. Being that the current standard for diagnosing PAM is via the cerebrospinal fluid, we also aimed to identify other less invasive routes that could be utilized in a point-of-care situation for diagnostic purposes. Extracellular vesicles (EVs) are small lipid-bound carriers that have been touted as fundamental players in cellular communication as they are capable of stably transporting materials through many different bodily fluids. One class of cargo known as microRNAs, which are small secretory molecules that can influence biological processes and gene regulation, have been increasingly used as biomarkers for human diseases such as cancer. Considering this, we hypothesized that smallRNAs secreted by Naegleria could hold the same potential. We performed deep-sequencing on the RNA contents of Nf-secreted EVs followed by bioinformatic analyses to uncover tRNAs, microRNAs, and other smallRNAs. With this initial insight into the types of smallRNAs secreted by Nf, we then focused on the prevalence of the RNAs which led to the identification of a highly prevalent smallRNA that we named Nf-smallRNA-1. Thus far, we have used qPCR to validate the presence of this smallRNA in EV preparations from 6 different clinical isolates. We have also detected this smallRNA in the plasma collected at the end-stage of infection in >70 mice infected with 7 different clinical isolates of N. fowleri. This and future work could provide a novel, less invasive option for diagnosis of PAM that could be implemented on fluids collected from patients at initial admission to the hospital and thus recover valuable time that is needed to save lives.

O4.4

Molecular analysis unmasking a *Balamuthia mandrillaris*: Skin lesion and ranulomatous amebic encephalitis by *Acanthamoeba sp* close to genotype T4 with fatal outcome

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Keywords: Acanthamoeba, Meningoencephalitis, Peru

Acanthamoeba and *Balamuthia* are groups of amoebae unrelated to *Naegleria*, but also free-living. Some species from this genus are infectious, causing two diseases. **Granulomatous amoebic encephalitis** (GAE) is a brain infection somewhat different from **Primary amoebic meningoencephalitis (**PAM). GAE occurs in immune-suppressed patients, usually secondary to infection elsewhere in the body (e.g. skin ulceration) Most cases are fatal after a long time illness. *Acanthamoeba* cysts and trophozoites are common all over the pathological lesions, while amoeba trophozoites are found in acute necrotic and inflamed areas, especially around blood vessels, whereas cysts are more concentrated in necrotic tissue.

Acanthamoeba sp, Balamuthia mandrillaris, are a free-living soil amoeba, has emerged as a causative agent of chronic GAE.

The patient had a skin lesion and that it behaved as if it were a case for *Balamuthia* mandrillaris, but our molecular analysis demonstrated by phylogenetic analysis that species corresponds to *Acanthamoeba* T4.

The information from the cases It's in the website university Alabama: https://www.uab.edu/medicine/gorgas/cases-blog/2019/2019-case-7

O4.5

Acanthamoeba castellanii: Inhibition of protease activities and cytopathic effect by bovine apo-lactoferrin

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Keywords: Acanthamoeba castellanii, bovine apo-Lf, proteases

Acanthamoeba castellanii is a free-living amoeba, which has an important clinical relevance causing granulomatous amoebic encephalitis and amoebic keratitis in human beings. During the initial steps of infection, the trophozoites interact with different host immune responses in the corneal epithelium, nasal mucosa and blood, such as lactoferrin (Lf). The evasion of innate immune response is crucial in the colonization process; however, Lf has an important role in the elimination of pathogenic microorganisms. In this work, we describe the resistance of *A. castellanii* to microbicidal effect of bovine apo-lactoferrin (apo-bLf) at different concentrations (25, 50, 100, and 500 µM). *A. castellanii* trophozoites incubated with apo-bLf at 500 µM during 12 h maintained the viability in 98%. Interestingly, our results showed that apo-bLf inhibited the cytopathic effect of *A. castellanii* on MDCK cell culture; the analysis of amoebic proteases showed an important inhibition of cysteine and serine proteases by the interaction with apo-bLf. With these results, we conclude that bovine apo-Lf has an effect on the activity of *A. catellanii* secretion proteases, which has an effect on the decrease of amoebic cytopathic activity.

O4.6

Pathogenicity of *Naegleria fowleri* isolates varies significantly in the mouse model of primary amoebic meningoencephalitis

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Keywords: Naegleria fowleri, virulence, pathogenicity

Naegleria fowleri, colloquially known as “brain-eating amoeba”, causes an acute, fatal disease called primary amoebic meningoencephalitis (PAM). N. fowleri is commonly present in warm freshwater and soil and although PAM is a rare disease, it results in >97% mortality rate. One persisting question is why so few people succumb to disease when so many are potentially exposed? We hypothesized that clinical isolates vary in inherent virulence and these variabilities affect the minimum infectious dose required to induce PAM. Utilizing a mouse model of PAM, we intranasally inoculated clinical isolates of N. fowleri (Nf69, V067, V631, Villa Jose, and V596) at three concentrations per mouse: 100, 1000, and 5000 amoebae. Results showed significant variability in onset of severe disease and mortality rates among isolates and within genotypes. The highest infectious dose (5000 amoebae) induced 100% mortality by all isolates except V067 (87.5%), with a large variance in onset of endpoint symptoms from 4 days post inoculation (dpi) to > 20 dpi. Remarkably, for isolate V596, only 100 amoebae produced 100% mortality by dpi 4-5 and we observed deaths with as few as 10 V596 amoebae. Concurrently, we assessed in vitro pathogenicity by comparing feeding rates among isolates seeded onto Vero cells. We observed variability in feeding rates for 12 N. fowleri isolates and although not all isolates were tested in vivo, our data suggest a positive correlation between an increased feeding rate in vitro and increased virulence in vivo. Overall, these results support our hypothesis of inherent differences in pathogenicity between isolates that result in variance in minimum infectious doses. We acknowledge support from US NIH (R03AI141709 to DEK and 5T35OD010433-14 to ES) and the Georgia Research Alliance (to DEK).

Session V: treatment and disinfection against free-living amoebae

Chairpersons: Christine Imbert - Dennis Kyle

Keynote speaker: Nicole Carnt (Sydney, Australia)

Acanthamoeba Eye Infection: A Clinical Researcher Perspective

O5.1

Activity of a Novel Marine Marolide against Different Strains of Acanthamoeba castellanii

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Keywords: Acanthamoeba, free-living amoeba, natural product

One of the most important free-living amoebae Acanthamoeba is an opportunistic water-borne parasite capable of infecting humans. When it enters through the eye, it can cause keratitis, resulting in blindness or poor visual impairment. The number of cases of this painful disease has increased over the past decades, usually in contact lenses users with improper cleaning habits of their lenses or with previous corneal trauma. Current treatments for Acanthamoeba keratitis involve chlorhexidine and polyhexamethylene biguanide (PHMB), which are effective at killing trophozoites, but cannot be tolerated at high doses limiting their effectiveness. Despite advances in combination therapies and surgery, cyst resistance to therapeutic agents and recurrence of infection remain a challenge that is yet to be addressed. Recently, we screened a subset of compounds available in the Repurposing, Focused Rescue and Accelerated MEdicinal chemistry (ReFRAME) library against trophozoites of Acanthamoeba castellanii and identified the trophocidal activity of a novel marine macrolide. We tested this marine natural product-derived compound against trophozoites of multiple strains and determined an EC50 that ranged between 0.6 and 2.2 μM. The compound was then evaluated against cysts of different strains and we evaluated the cysticidal activity at different concentrations over a number of days. To test whether the marine macrolide influences invasiveness of A. castellanii, we used a transwell matrigel invasion assay. A 4 h pre-incubation of trophozoites of two strains with the compound at its EC50 concentration decreased the invasion of trophozoites significantly after 48 h compared to 0.5% DMSO-treated trophozoites. Considering the availability of eyedrop formulation and completion of Phase I clinical trial of this compound, this marine natural product-derived compound warrants further investigation of as a new topical agent in the treatment of Acanthamoeba keratitis.

O5.2

Pitavastatin loaded nanoparticles: Disruption of cytoskeleton structure and induction of cell death via autophagy in *Acanthamoeba polyphaga*

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Keywords (Acanthamoeba spp, Polymeric Nanoparticles, Pitavastatin)

Pitavastatin is a lipid lowering agent belonging to the third Statin generation. Statins were reported to lower the plasma low-density lipoprotein cholesterol (LDL-C) concentration by inhibiting the 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase. Lately, pitavastatin has been reported to exhibit anti-tumoral, anti-inflammatory and anti-*Acanthamoeba* effects (1,2). However, it remains underused mainly in aqueous formulation due to their poor hydro solubility. The aim of the present study was to develop Pitavastatin loaded nanoparticles suitable for ophthalmic administration and designed for the management of *Acanthamoeba* Keratitis. These nanocarriers would represent a solution to both the administration-route associated problems and the limited aqueous drug solubility issues. Nanoparticles were obtained by a nanoprecipitation-solvent displacement method and their amoebicidal activity was evaluated against four strains of *Acanthamoeba*: *A. castellanii* Neff, *A. polyphaga*, *A. griffini* and *A. quina*. Later, the effect of the pitavastatin nanocarriers was investigated with respect to the microtubule distribution and several program cell death features in *Acanthamoeba polyphaga* using Fluorescence and electronic microscopies. The effect of Pitavastatin-loaded nanoparticles on the tubulin and actin distribution was examined using a direct staining and an indirect immunofluorescence assay. After 48 hours of incubation with the nanoparticles, a highly reduced cell shape was observed with an uniformly distributed tubulin and actin which emit lower fluorescence than the negative control. In addition, the present nanocarriers could induce several autophagic and apoptotic clinical features including the formation of autophagic vacuoles, chromatin condensation and collapse of the mitochondrial membrane potential. Both types of cell death could be due to the accumulation of reactive oxygen species and oxidative stress. The present pitavastatin formulation could inhibit *Acanthamoeba polyphaga* by affecting first its motility and later by inducing programmed cell death.

O5.3

Development of a Machine Learning-based Assay and Identification of Amoebicidal Marine Microbial Metabolites against *Acanthamoeba castellanii* and *Naegleria fowleri*

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Keywords: Acanthamoeba, Naegleria, free-living amoeba

Free-living amoebae are causes of morbidity and mortality associated with keratitis and meningoencephalitis. Primary Amoebic Meningoencephalitis (PAM), caused by a free-living amoeba Naegleria fowleri, has a fatality rate of over 97%. Painful blinding keratitis is caused by the free-living amoeba Acanthamoeba and can occur in healthy individuals wearing contact lenses. There are no FDA-approved drugs to treat PAM. Current treatment for Acanthamoeba keratitis relies on a combination of chlorhexidine, propamidine isethionate, and polyhexamethylene biguanide. However, in 10% of cases recurrent infection ensues, because of the difficulty in killing both trophozoites and double-walled cysts. Therefore, development of efficient drugs is a critical unmet need to avert blindness and future deaths. The current standard for screening compounds for cysticidal activity is to treat Acanthamoeba cysts with a compound of interest and manually observe for evidence of excystation, such as proliferating trophozoites or distinctive trails left in agar media by trophozoites. These cysticidal screening techniques are labor-intensive and low-throughput, which limits their utility and efficiency. Automating cysticidal assays would significantly enhance current screening capabilities for Acanthamoeba and speed up the rate of discovering cysticidal compounds. To automate and modernize cysticidal drug screens, we adapted and trained a machine learning object-detection neural network to recognize A. castellanii trophozoites and cysts in microscopy images. We utilized this trained neural network as a tool to count excysted trophozoites in treated wells to determine if a compound treatment was cysticidal. We undertook a large-scale bioluminescence-based screen of structurally unique marine microbial metabolites against the trophozoites of both A. castellanii and N. fowleri. We identified about 100 and 29 trophocidal hits against N. fowleri and A. castellanii and subjected the 29 identified trophocidal hits to this machine learning-based automated Acanthamoeba cysticidal assay. One marine microbial metabolite fraction was identified as both trophocidal and cysticidal. These fractions may harbor anti-Acanthamoeba or anti-Naegleria molecules that would serve as attractive drug development molecules. Based on these, we will purify the active metabolites, and investigate the pure compounds for their effect on both Acanthamoeba and Naegleria.

O5.4

Discovery of Acanthamoeba Trophocidal and Cysticidal Agents using a Novel High-Content Screening Method.

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The pathogenic free-living amoeba, Acanthamoeba, cause several diseases including a severe brain disease known as Granulomatous Amoebic Encephalitis (GAE), with >90% mortality rate. Acanthamoeba more commonly causes Amoebic Keratitis (AK), in association with poor contact lens hygiene or behaviours, which can result in blindness. The current drug regimens were not originally discovered for Acanthamoeba infections but have shown moderate in vitro or in vivo potency against these organisms. The unoptimized therapy, long treatment duration, and potential relapse of keratitis infections indicate these therapeutic regimen(s) are not the best indicative treatments for current or future patients. New specifically developed drugs for these diseases need to be discovered, developed, and implemented into the current drug regimen(s) to give patients a better chance of preventing sight loss. Herein, using high-throughput screening (HTS) and a newly developed high-content screening (HCS) method, we screened over 400 compounds that contain bis-benzimidazole amidine and diamidine scaffolds. We discovered several compounds which possessed nanomolar or low micromolar anti-trophozoite activity and prioritized only the nanomolar inhibitors for cysticidal activity assessment. After applying an SAR approach in our drug discovery pipeline, forty-two compounds showed cysticidal or recrudescence inhibitor activity against three clinical isolates of Acanthamoeba tested at 6 hours of drug exposure with persistence and recrudescence assessed for up to 30-days. The compounds we developed are more potent than any other known cysticidal agents currently described in the literature. Also, this is the first development of a novel high-content screening method to validate and verify cysticidality/recrudescence inhibition of Acanthamoeba species.

O5.5

Cyanomethyl Vinyl Ethers against *Naegleria fowleri*

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Keywords: Naegleria fowleri, Primary Amoebic Meningoencephalitis, Programmed Cell Death

Naegleria fowleri is the causative agent of a fulminant disease affecting the central nervous system called primary amebic meningoencephalitis. The disease is fatal in more than 97% of reported cases, mainly affecting children and young people who reported having been involved in aquatic activities in fresh and warm non-treated water bodies that were contaminated by this pathogen. Currently, treatment of primary amoebic meningoencephalitis is based on a combination of different antibiotics and antifungals, which, however, are not entirely effective and lead to numerous side effects. For this reason, this study analyzed the amebicidal activity of 46 Cyanomethyl Vinyl Ethers against two strains of Naegleria fowleri (ATCC® 30808 and ATCC® 30215). The compounds that showed most activity and less cytotoxicity were QOET-51, QOET-59, QOET-64, QOET-67, QOET-72, QOET-77 and QOET79. All of them had selectivity index values (CC50/IC50) greater than 6, even higher than some reference drugs. In addition, these products were assayed in mechanism of action assays to demonstrate the type of programmed cell death produced in Naegleria fowleri. The results indicated that QOET-59, QOET-72 and QOET-77 induced programmed cell death process in the treated amoebae, causing events such as DNA condensation, plasma membrane damage, reduction of mitochondrial membrane potential and ATP levels, generation of ROS or disassembly of the cytoskeleton. Thus, Cyanomethyl Vinyl Ethers could be considered as a potential novel therapeutic agent for the development of treatments against primary amoebic meningoencephalitis in the near future.

O5.6

Mechanisms of quaternary ammonium compound resistance in *Acanthamoeba castellanii* Trophozoites

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The sight-threatening infection, Acanthamoeba keratitis, is normally associated with contaminated contact lens wear as the lenses can promote small tears in the corneal surface allowing entry of the pathogen. At present, there are no effective 'Acanthamoebocides' available for disease treatment or prevention and most current treatments induce the transformation of Acanthamoeba active trophozoites into a highly resilient dormant cyst. Some quaternary ammonium compound (QACs) analogues have significant biocidal activity against cysts strongly linked with structural variations. However, suboptimal levels over prolonged periods promote resistance. This approach was used to generate QAC-resistant trophozoites, and biochemical characterisation shows that it is achieved by biodegradation of the compound. Similar pathways are involved in antibiotic resistance. The emergence of antimicrobial resistance (AMR) is a significant threat to anti-infective treatments and this observation offers valuable insight to how AMR develops in Acanthamoeba. Here, we present data from a comparative study aimed to elucidate the mechanistic route used by the protist to develop resistance to the active QACs. The results from this study will enable the eye-care and pharmaceutical industries to remain one step ahead in the clinical arms race against Acanthamoeba, safeguarding the effectiveness of QACs as a medical device for the prevention of Acanthamoeba infections in contact lens wearers.

O5.7

Gongolarones as antiamoeboid chemical scaffold

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Keywords: Acanthamoeba spp.; Programmed Cell Death; meroterpenoid.

The use of molecules produced by marine organisms to treat several diseases has notably increased in the recent years. In this sense, current studies focus on research to find new compounds with natural origin and amoebicidal activity for the treatment of *Acanthamoeba* infections [1,2]. Among these marine organisms, macroalgae contains a wide variety of bioactive secondary metabolites with diverse pharmacological properties. Brown algae, such as *Gongolaria abies-marina* (previously *Cystoseira abies-marina*, S.G. Gmelin), have been shown to have interesting bioactive molecules, including antiprotozoal activity. In this study, six meroterpenoids (**Gongolarones A**, **B** and **C**; cystomexicone B, 1'-methoxyamentadione and 6Z-1'-methoxyamentadione), were isolated and purified from the alga *Gongolaria abies-marina*.

All isolated compounds exhibited amoebicidal activity against *Acanthamoeba castellanii* Neff, *A. polyphaga* and *A. griffini*. Gongolarones **A** and **C** showed the lowest IC_{50} values against two stages of tested strains. Furthermore, structure-activity relationship (SAR) reveals that the cyclization by ether formation from C-12 to C-15 of **A**, and the isomerization □2t to □3t of **C**, increases the antiamoeboid activity of both compounds. Gongolarones **A** and **C** triggered programmed cell death (PCD) in trophozoites of *Acanthamoeba* tested in this study, showing chromatin condensation, mitochondrial damage, and oxidative stress remarkable by the cell ROS production. Additionally, novel compounds **A** and **C** disorganized the actin and tubulin networks of treated trophozoites. Finally, transmission electron microscopy (TEM) images analysis revealed that compounds **A** and **C** induced autophagy process and inhibited the encystation process in *A. polyphaga* trophozoites.

Taking all the results into account, seaweeds including *Gongolaria abies-marina* could be a source of bioactive marine molecules for developing new antiamoebic compounds against *Acanthamoeba* infections.

O5.8

Synergistic effect of auranofin and hydrogen peroxide, chlorhexidine and polyhexamethylene-biguanide on clinical isolates of Acanthamoeba genotype T4.

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Keywords: Acanthamoeba, therapy, auranofin

Prevention and treatment of *Acanthamoeba* keratitis (AK) remains a challenge despite advances in antimicrobial therapy and better understanding of anti-*Acanthamoeba* disinfection. Currently, treatment of AK involves a combination of biguanides such as polyhexamethylene-biguanide (PHMB) or chlorhexidine and diamidines, such as hexamidine isethionate, dibromopropamidine, and propamidine isethionate. Due to their toxicity to corneal cells, these agents have to be applied hourly in low concentrations during the first days of therapy, followed by frequent application for up to six month. When started early, this treatment regimen is effective against trophozoites and cysts, however, the outcome depends on the patients' compliance and recurrent infections are still common.

Auranofin is a gold-based compound that interacts with the thioredoxin system and leads to cellular oxidative stress and intrinsic apoptosis. Auranofin has a well-known toxicity profile, is well-tolerated and has been proposed as an ideal candidate for drug repurposing. It has been shown to be active against various bacteria and several protists, including *Entamoeba histolytica*, *Giardia duodenalis* or *Naegleria fowleri*. Also in *Acanthamoeba* spp., promising amoebicidal effects have been demonstrated, however, requiring prolonged incubation times.

The aim of is study is to evaluate potential synergistic effects of auranofin and compounds applied in *Acanthamoeba* therapy or disinfection. Different concentrations of auranofin in combination with chlorhexidine (CHX), hydrogen peroxide (H_2O_2) and PHMB are tested for their effect on several clinical *Acanthamoeba* isolates. The amoebicidal effect is determined by Trypan Blue staining and enumerating dead amoebae with a haemocytometer.

Our preliminary results show that the combination of auranofin with all tested compounds leads to a significant synergistic effect. This was particularly pronounced for the combination of auranofin and CHX, even within a very short incubation time of the latter. Additionally, the incubation time for auranofin could be significantly reduced compared to previous observations. The next step will be to evaluate the most effective combination, considering both incubation time and concentration of compounds.

Altogether a combination of auranofin and other compounds might enable a more effective and less tortuous option for the therapy of *Acanthamoeba* infections.

O5.9

Chamigrenes isolated from the Laurencia obtusa against *Naegleria fowleri*

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Primary amoebic meningoencephalitis (PAM) is a central nervous system affecting disease caused by the opportunistic protozoa Naegleria fowleri. It is a fulminant disease with a rapid progression and affects mostly children and young adults who reported previous exposure to warm water bodies, such as lakes, hot springs or untreated swimming pools. The trophozoite stage of this amoeba penetrates the nasal cavity and migrates through the olfactory nerves to the brain causing infection and subsequent death during the first 18 days after the onset of the first symptoms. Currently available therapeutics are not 100% efficient and are related to a wide variety of severe side effects. Therefore, the search for novel molecules with anti-Naegleria activity and low toxicity remains an urgent issue in the fight against the PAM.

On the other hand, the red algae of the Laurencia genus have been proved to be an important source of bioactive molecules. In fact, some compounds obtained from different red algae namely Laurencia viridis or Laurencia johnstonii have shown great activity against diverse protozoa, including some parasites that belong to the free-living amoeba group. In this study, the anti-Naegleria activity of different molecules obtained from the Laurencia obtusa algae was evaluated.

As a result, the chamigrenes (+)- elatol, (+)- obtusol and -(-) elatol were the most active molecules against Naegleria fowleri trophozoites. Moreover, the type of cell death process that produces the (+)- elatol, the most active compound, was also determined, showing a programmed cell death induction in treated cells. Hence, (+)- elatol could be considered as a good candidate for the development of new treatments against the primary amoebic meningoencephalitis.

O5.10

Evaluation of the activity of cerivastatin and other statins against *Acanthamoeba*

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Keywords: Acanthamoeba, statins, chemotherapy.

Acanthamoeba spp. is a group of amphizoid protozoa widely distributed in the environment, which are part of the so-called Free-Living Amoebas (FLA). This genus represents a potential risk to human health due to its ability to cause diseases, such as Acanthamoeba Keratitis (AK) or Amoebic Granulomatous Encephalitis (GAE). These organisms present two phases in their life cycle, a vegetative stage called trophozoite and a cyst stage, where metabolic activity is minimal, allowing them to cope with adverse conditions.

Currently, there is no fully effective treatment against *Acanthamoeba* and those used to date have high toxicity. This has prompted the search for active principles that simultaneously present high amoebicidal capacity and low toxicity for the host. The objective of this study is to evaluate the activity of different statins against two clinical strains of *Acanthamoeba* and the effects induced at the cytoskeleton level and programmed cell death events.

The *in vitro* activity against *Acanthamoeba* spp. trophozoites was based on a fluorometric assay using the alamarBlue® reagent. To carry out the study of the mechanisms of action, different commercial kits were used, following the manufacturer's instructions, such as the SYTOX Green kit and the Hoechst 33342/PI kit. Based on the results obtained, cerivastatin was the most active compound (IC50 of 1.70 ± 0.19 µg/ml for *A. culbertsoni* 0.28 ± 0.09 µg/ml for A. L-10). The mechanisms studied indicated that this third-generation hydroxy-methyl-glutarylco-enzyme A (HMG-CoA) reductase inhibitor promotes programmed cell death in both *Acanthamoeba* strains and disrupts their cytoskeleton. Therefore, the statins tested, and specifically cerivastatin, are good candidates for use as a future treatment.

Posters

P01

Influence of two aquatic macrophytes on Free-living amoebae assemblages composition in a shallow channel

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Keywords: Ecology, Macrophytes, Freshwater.

Free-living amoebae (FLA) can be affected by some biological factors, including the presence of organisms like aquatic macrophytes, which have the potential to inhibit the amoebae or give them adequate conditions to survive, such as food, habitat, and protection. It is not well known how the interaction between FLA and environmental conditions works. The study of FLA has been focused on a few species with medical concerns, and the ecological study of the non-pathogenic FLA has been neglected. For this reason, the present research aimed to analyze how the distribution and abundance of FLA are related to the presence or absence of two different macrophytes.

Two samplings were made in three different sites covering the rainy and dry seasons; the study site is a channel bordering a shallow lake (Salazar Lagoon) at a high altitude (3000 m a.s.l.) in Central Mexico. The first collecting site was one with *Potamogeton* sp. (a submerged macrophyte), the second with *Hydrocotyle* sp. (emerged macrophyte), and the third without plants. Water temperature, conductivity, dissolved oxygen, pH, chlorophyll *a*, total bacteria counts, total phosphorus, and total nitrogen were measured to relate them with the amoebae distribution and abundance.

Fifteen taxa belonging to eleven genera were identified. *Arcella*, *Naegleria*, and *Vermamoeba* were observed in all sites at both seasons. *Vannella* and *Korotnevella* were observed only in the dry season while *Difflugia*, *Saccamoeba*, and *Thecamoeba* were only in the rainy season. The two sites with macrophytes had eleven taxa each, meanwhile, the site without macrophytes only had eight. *Naegleria* was present in all sites in both seasons. Naked amoebae abundances had a significant correlation with total bacteria counts (p=0.058). The abundance of naked amoebae was lower than the testate, and the site with *Hydrocotyle* had the highest amoebae abundance.

In conclusion, the effect of the macrophytes on the amoebae is not direct, even though the macrophytes can affect amoebae indirectly by changing the environment around them, giving them more complexity, changing the environmental parameters, or the food availability.

P02

Presence of potentially pathogenic free-living amoebae in aquatic environment related with human activities

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Keywords: free living amoebae, human risk management, environmental health

The study was carried out in selected European countries: (Czechia, Slovakia, Serbia, Albania, Cyprus, Greece, Azores) during 2022. The collection of strains FLA isolated from 33 sampling sites was determinate by cultivation and termotolerance. The aim of our work will be to confirm the possible pathogenicity of selected strains of environmental isolates by molecular analyzes. The FLA of Acanthamoeba and Vermamoeba genera were most often identified by our cultivation and microscopic observation. The fact that they are most often isolated representatives of FLA should be noticed and provoke a discussion with a multidisciplinary approach in solving this problem.

With our results we want to support improvement of procedures for identification, assessment and evaluation of the severity of environmental and human risks linking to importance and the role of FLA as vector of microorganisms, identified several endosymbionts, not only their spread to the environment but related to rising their virulence and pathogenicity.

The work will provide a comprehensive overview of management procedures needed to prevention and to avoid extraordinary incidents and to solve their impacts on the environment and public health through a practical solution proposal.

P03

Successful cultivation of *A. castellanii* from Acanthamoeba keratitis mouse model

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Keywords: Acanthamoeba; AK mouse model; cultivation

Acanthamoeba is a free-living amoeba that can cause granulomatous amebic encephalitis (GAE) or an eye infection referred to as Acanthamoeba keratitis (AK). AK occurs mainly in contact lens wearers such as long-term use of lens, wrong contact lens ringing and corneal trauma. In vitro studies of AK, especially the development of therapeutic drugs, AK finding needs to be confirmed by in vivo experiments. We have established an AK mouse model in a previous study. *A. castellanii* (5 x 10^4 cells) were loaded onto 2 mm contact lens pieces for insertion into mouse scratched eyes under anesthesia, and the eyelids of the mice were sutured. After infection (1 to 14 days), in the daily follow up, it was observed that the AK lesion had progressed in mouse eyes, and PCR confirmed the amplification of the Acanthamoeba DNA. In this present study, we investigated the effective cultivation method in order to certified PCR analysis in the AK mouse model. After inoculation (1, 3, 7 and 14 days) as according to previous methods (PCR amplified), eyeball samples were obtained from the AK mouse model. Successful cultivation from homogenized eyeball of *A. castellnii* were done in the in the non-nutrient agar with the lawn of *Escherichia coli*, and scaled up with PYG medium. In addition, PCR products amplified from the AK model-cultured amoeba and the genetic information of the inoculated amoeba was matched (> 99 %). We propose that the cultivation and PCR method may be used for the confirmation of AK development in AK mouse model.

P04

Development of acanthamoeba keratitis in mouse induced by *Acanthamoeba castellanii* pretreated with commercial contact lens solutions

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Keywords: Acanthamoeba; Acanthamoeba keratitis; Ttreatment

Ubiquitous *Acanthamoeba castellanii* causes the acanthamoebic keratitis (AK) in mainly in contact lens wearers. Because the early diagnosis and treatment of AK is not easy, the prevention, including personal hygiene and the use of appropriate solutions, is important. For the efficacy of two commercial contact lens solutions, in this study, the in vitro amoebicidal effect examined after pre-treatment with O-F/00 or r-n/00 solutions. Then whether they could delay or suppress the AK occurrence in experimental AK mouse model carried out. After treatment with each 50% diluted solution in amoebic culture system, *A. castellanii* changed into round-form pre-cysts from 6 hr and not dead until 24 hr. When each 100% solutions were treated, a few dead amoebae founded from 24 hr. In mice inoculated with *A. castellanii* pretreated with each 100% of O-F/00 and r-n/00 solutions for 1 and 6 hr, *A. castellanii* showed distinct AK induction from day 1, as similar to those of positive control groups. *A. castellanii* pretreated for 12 hr did not induce clearly AK on day 1, but a distinct AK sign revealed from day 2 or 3. AK signs induced by *A. castellanii* pretreated with O-F/00 and r-n/00 solutions for 24 hr were not clear on day 1 or 2, but a pronounced AK sign showed from day 3 or 5 after infection. Additionally, all experimental AK development confirmed by PCR for18S-rDNA amplification from mouse eyeball DNA samples. Finally, it is thought that treatment with two contact lens solution have the effect on delaying the occurrence AK a little.

P05

Identification of antigens from *Naegleria fowleri* recognized by serum and saliva antibodies from people of Mexicali Valley, Mexico

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Key words: Naegleria fowleri, antigens, antibodies

Naegleria fowleri is an amoeba that causes a fatal disease in the central nervous system known as primary amoebic meningoencephalitis (PAM) in humans. Most of the infections are acquired by people who practice recreational activities in water contaminated with trophozoites. Swimming and wading in irrigation channels of Mexicali are common practices for local people. Although there are some warning legends in the surrounding sites, people continue using these channels for recreational purposes. In that area, cases of PAM have been reported but the immune response against *N. fowleri* from these people is unknown. Thereby, we analyze IgA, IgG, and IgM antibodies level against *N. fowleri* as well as we identified antigens recognized by these antibodies. Serum and saliva samples were obtained from subjects and the specific antibodies response was analyzed by immunoassays (Western blot, ELISA and cytochemistry). We also applied a protein analysis to detect immunogenic antigens that were recognized by people antibodies. We found the presence of specific IgG, IgA and IgM antibodies against different polypeptide bands from *N. fowleri* (50, 42, 37, 30, 25 and 19 kDa). The antigens present in these bands match those already have been reported as immunogenic in our mice protection model against PAM. Moreover, antibodies from some subjects recognized trophozoites surface with great intensity. Antibodies from local people that recognize *N. fowleri* antigens could be induced by a constant stimulus with *N. fowleri* antigen in the area or through a cross-reaction stimulated by other species of amoebas. This antibody immune response along with other factors could be participating in the defense of these people against PAM.

P06

Utilization of varying techniques to portray the intracellular interactions and extracellular vesicles secreted by *Naegleria fowleri*

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Keywords: Naegleria fowleri, pathogen-host interactions, scanning electron microscopy

The free-living amoeba, Naegleria fowleri, causes primary amoebic meningoencephalitis, an acute brain disease with a case mortality rate of >97%. Several factors contribute to this considerable degree of mortality including delayed diagnosis, ineffective therapeutics and lack of understanding of the amoebic pathogenesis. The understanding of the molecular basis for parasite-host interactions is also lacking, thus, we endeavored to unravel the mechanistic basis used by N. fowleri in relaying cell-cell communications. Extracellular vesicles (EVs) are small membranous vesicles from the endosome or plasma membrane that have been implicated as mediators of intercellular communication. EVs are secreted by originating cells and contain signaling molecules that elicit a response in recipient cells. We hypothesized that N. fowleri produces EVs and packages proteins and other cargo into them to be secreted and elicit a response in recipient cells. To test this hypothesis, secreted components from amoebaconditioned media were extracted and purified followed by enumeration and size determination with Nanoparticle Tracking Analysis. Mass spectrometry analyses of EV suspensions have also identified thousands of proteins associated with the secreted vesicles. To measure EV uptake, fusion assays using the self-quenching R18 dye show that these EVs are taken up by host-cells and other amoebae. Subsequent real-time viability testing using the RealTime-Glo MT Cell Viability Assay indicates an increase in reducing potential across multiple mammalian cell lines when exposed to Nf EVs. To visually inspect the EVs, scanning electron microscopy not only confirmed EV morphology/size, but also provides potential routes through which amoebae secrete these vesicles, and show instances of intracellular interactions via pseudopodia and filopodia that potentially allow for signaling or material exchange. This as well as future work will expand the knowledge of the intracellular interactions among these amoebae and subsequently deepen the understanding of the mechanistic basis of PAM.

P07

Presence of potentially pathogenic free-living amoebae in the water distribution network for human use in the Tehuacan-Cuicatlan Valley, Mexico

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Key words: intertropical desert, Acanthamoeba, water quality

Tehuacan-Cuicatlan Valley is surrounded by mountain ranges, archeological discoveries in the valley are 7,000 years old, this period corresponds with the domestication of corn and the sedentary lifestyles. Tehuacan has location and climatic conditions create high diversity and endemism, home of the oldest water management system in North America. Unfortunately, the inappropriate treatment of the urban, industrial, and agricultural water supply affects the people quality life exposing them to dangerous health risks. Drinking poor-quality water can lead the transmission of diseases and it is essential to know the water condition provided by the Valley, which is known for not having potabilization and wastewater treatment. The objective of this work was to determine the presence of potentially pathogenic free-living amoebas (FLA) in distributed sites in Tehuacan-Cuicatlan. Water samples of 250 mL were collected in five environments, to which hydrochemical parameters were measured and it was done bacteriological evaluation by NMP/100 mL. Water samples were filtered throught cellulose nitrate filter (1.2 µm); we placed inverted on non-nutritive agar plates seeded with *Enterobacter aerogenes* (NNA), then were incubated at 30º and 37°C. The plates were observed to detect amoebae growth and were subcultured in obtain axenic cultures. Morphological identification of isolates was performed using taxonomic keys. The results shown that physico-chemical parameters were: pH of 7.1 to 7.4, orthophosphates 1.1 to 1.4 mg/L, Si 9 to 11 mg/L, $NO3^-$ 2.1 to 2.4 mg/L, MgCO3 6.5 to 6.9 g/L, CaCO3 0.032 to 0.036 g/L, Na_2CO3 118 to 120 g/L, MgSO4 205 to 209 g/L, CaSO4 1 to 1.2 g/L, Na_2SO4 65 to 68 g/L, CaCl2 5 to 7 g/L, MgCl2 94 to 98 g/L, NaCl 190 to 198 g/L, dissolved oxygen 1.5 to 3.1 mg/L. The bacteriological evaluation resultedgave as a result 1100 NMP/100 mL. *Acanthamoeba polyphaga, A. tubiashi, A. hatchetti, Vermamoeba vermiformis* y *Naegleria fowleri* sp were found. It is the first time that these findings have been found in terms of water quality in the study area, representing a risk to the health of the system and to the residents, who can help raise awareness to implement water purification and wastewater treatment.

P08

Evaluation of the inhibitory effect of non-steroidal anti-inflammatory drugs on the proliferation of *Acanthamoeba* spp.

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Keywords: Proliferation inhibition, NSAIDs, Acanthamoeba

Acanthamoeba spp. are free-living and opportunistic protozoa, capable of causing granulomatous amebic encephalitis and amebic keratitis; pathologies for which there is no treatment of choice. The cyclooxygenase/prostaglandin (COX/PG) axis was recently proposed as a therapeutic target due to the detection of prostaglandins in *Acanthamoeba* (1). In addition, the inhibition of the proliferation of *A. castellanii* by some non-steroidal anti-inflammatory drugs (NSAIDs) has been reported (4).

In this work, the inhibitory activity of different concentrations of diclofenac and two newly synthesized compounds were evaluated: COX-2 selective isoindoline 4, and COX-1 selective isoindoline 10 (2,3), on the proliferation of 1×10^4 trophozoites of *A. castellanii* (isolated clinical) and *A. culbertsoni* (ATCC 30171), during 24 h at 30ºC.The inhibitory concentration 50 (IC_{50}) was determined by the crystal violet viability technique.

Diclofenac inhibited *A. culbertsoni* proliferation 72% at a concentration of 600 µM, with an IC_{50} of 219 µM; the maximum inhibition determined *in A. castellanii* was 89% also with a same concentration and IC_{50} of 86 µM. The IC_{50} of isoindoline 4, obtained in *A. culbertsoni* was determined at 600 µM, and for *A. castellanii* the maximum inhibition observed was only 39% at a concentration of 1000 µM. Moreover, isoindoline 10, showed a low effect on the proliferation of both species since the maximum concentration evaluated of 1000 µM exhibited a maximum inhibition of 26.3 and 9.2% against *A. culbertsoni* and *A. castellanii,* respectively.

The proliferation inhibitory effect of the tested NSAIDs whose target molecule is COX-2, depended on the amoebic species evaluated; isoindoline 4 showed a greater effect on *A. culbertsoni*, while diclofenac induced greater inhibition on *A. castellanii*. However, the inhibitory concentrations are high, so it would be important to also evaluate its cytotoxicity in the target tissue at these concentrations. It is also important to determine if the inhibition of amoebic proliferation of these compounds involves the COX/PG axis or acts at the cell cycle level, which will provide information on the biology of these amoebae.

P09

Biochemical and molecular mechanisms in the kidneys of mice infected with *Acanthamoeba* sp.

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Keywords: kidneys, Acanthamoeba sp.

The main biotope of *Acanthamoeba* sp. is the brain and cornea. However, amoebas may migrate with the blood and invade the distant organs, such as kidneys. In parasitic diseases, renal dysfunction is often not reflected in host serum creatinine and/or urea levels. Exact kidney injury mechanisms in parasitic infections are poorly known in many cases, bringing major difficulties to specific therapeutic interventions. Additionally, kidney involvement in parasitoses is almost always late, being an important cause of medical complications. The aim of the study was to check which mechanisms are involved in kidney host response to amoebas.

Immunocompetent and immunosuppressed mice (n=96) were divided into two groups: *Acanthamoeba* sp. infected (n=60) and uninfected (n=36). Animals were intranasally inoculated with *Acanthamoeba* sp. (T16 genotype) and then sacrificed at 8, 16, and 24 days post infection (dpi). Based on the results of our studies, it is known that *Acanthamoeba* sp. activate the protein that play a key role in the innate immune system, Toll-like receptor 2 (TLR2), in the mouse kidneys. TLR2 activation significantly increased the synthesis of various cytokines and chemokines, including monocyte chemoattractant protein -1 (MCP-1) and transforming growth factor-β (TGF-β). These cytokines modulated the activity of extracellular matrix metalloproteinases -2 and -9 (MMP-2 and MMP-9). Changes in the levels of MMPs were also influenced by hypoxia (changes in hypoxia-inducible factor -1α and -2α, HIF1α and HIF2α, respectively) and dysregulation of apoptotic proteins (pro-apoptotic protein – Bax and anti-apoptotic protein *Acanthamoeba* sp. – Bcl-2). We also found that MMPs activated kidney injury molecule -1 (KIM-1) synthesis in the kidneys of *Acanthamoeba* sp. infected mice. The neutrophil gelatinase-associated lipocalin (NGAL) were also changed, and therefore, they can be used as biomarkers of kidney dysfunction in disseminated acanthamoebiasis. Histopathological examination of kidneys of *Acanthamoeba* sp. infected mice revealed lighter staining of some renal tubules and small inflammatory foci.

This is the basic scientific research. Understanding the pathomechanisms of infection through biochemical and molecular studies of the *Acanthamoeba* sp.-host system is essential to develop new diagnostic procedures and identify new therapeutic targets to reduce the degree of cell and organ damage in the future.

P10

Amoebae, vectors of pathogenic bacteria in coastal waters?

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In the last few years, different studies have shown that climate changes have various impacts on aquatic bacterial ecosystems, including their hosts and their pathogenic capacity (Cirillo et al., 1994, 1997; Hoque et al., 2021). In that context, free-living amoebae may play a major role as they are considered as reservoirs and training grounds for pathogens.

As part of the "One World, One Health" concept, this study aims first to determine if amoebae are vectors of pathogenic bacteria in coastal waters and second to assess the impact of environmental parametersin the amoeba-bacteria interaction, specifically with bacteria of the Vibrio, Mycobacterium and Legionella genera, which are potential pathogens for both humans and animals.

In order to evaluate the influence of seasonality on the populations of microorganisms, water samples were monthly collected along a salinity gradient from the Charente River, France to the Atlantic sea. Therefore, various physico-chemical parameters were measured (such as temperature, salinity and pH). Water samples were filtrated: 1) for isolating amoeba strains, 2) for extracting total water DNA (abbreviated twDNA).

Amoebae were isolated after culture on ASW (Artificial Sea Water) plate, with a varying salt concentration depending on the origin of the sample and enriched in Escherichia coli as food source. Each isolated amoeba was then identified by sequencing of 18S rRNA gene. In parallel, qPCR was performed to detect potential pathogenic bacteria.

Total water DNA was extracted and specific qPCR assays allowed the identification of commonly found disease-causing pathogenic bacteria, as well as amoebae commonly detected in aquatic environments, using genus- and species-specific primers.

Preliminary results are promising, validating the approach chosen to isolate and identify amoebae present in coastal waters and their associated bacteria.

P11

Free-living amoebae isolated from cooling water with high salt concentration.

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Key words: cooling water, high salt concentration, Acanthamoeba

Free-living amoebae can cause fatal brain infections in humans, but they are also can live in nature. Some of these amoebae may be present in extreme environmental conditions, among which the species of the genus Acanthamoeba stand out. Samples were collected from three channels and one lagoon of cooling water from a geothermal plant. Conductivity, temperature, pH, and dissolved oxygen of the water were measured in situ. Water samples were filtered through a 2.0 µm-diameter Millipore filter, membranes were placed on Petri dishes with a non-nutrient agar medium with Enterobacter aerogenes (NNA), plates were incubated at 37°C and examined through an inverted microscope to detect growth of free-living amoebae. Isolated amoebae were axenizated in Chang medium. A preliminar morphological identification were done to genus level. For the classification of the isolates at the genotype level, the diagnostic fragment 3 of the 18S rDNA gene was amplified. The obtained products were purified and sequenced and the DNA sequences were compared to the ones available in the Genbank database. All isolated strains belonged to Acanthamoeba genus and phylogenetic analysis revealed that four strains belonged to genotype T10 Acanthamoeba culbertsoni and two strains belonged to genotype T5 Acanthamoeba lenticulata. Only the strains of A. culbertsoni were pathogenic when they were inoculated in mice. Lagoon parameters were temperature of 19 to 22 °C, pH of 6.6 to 6.7, dissolved oxygen of 5.0 to 5.8 mgL-1 and conductivity of 4.1x 104 to 5.2x104 µScm-1; channels parameters were temperature of 34 to 54 °C, pH of 7.3 to 8.1, dissolved oxygen of 1.2 to 2.2 mgL-1and conductivity of 4.0x104 to 4.4x104 µScm-1. Conductivity of cooling water was like the conductivity of sea water (5.0 x104 µScm-1). Naegleria has been reported in cooling water, but in this research was not present due to high salt concentration. The occurrence of species of Acanthamoeba in the cooling water with high salt concentration showed the capacity of the amoebae of this genus to stand extreme environmental condition. The occurrence of pathogen Acanthamoeba is a risk to the health of the workers of geothermal plant.

P12

Inhibition of the selenoprotein thioredoxin reductases in the genera *Naegleria* and *Acanthamoeba*

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Keywords: treatment, auranofin, thioredoxin reductase inhibitors

The genera Naegleria and *Acanthamoeba* are two of the four genera of free-living amoebas that are able to infect humans[1]. Among all *Naegleria* species only *N. fowleri* is pathogenic causing Primary Amoebic Meningoencephalitis (PAM) which is an acute fulminant, necrotizing and haemorrhagic meningoencephalitis. PAM occurs world-wide and it is a rare disease, but an increase in the number of cases has been reported. It occurs in immunocompetent children and young adults and its fatality rate is high (96 %), mainly because of the delay in initiation of treatment due to late diagnosis and unsatisfactory treatment options[2,3]. On the other hand, several species of *Acanthamoeba* are opportunistic pathogens causing a sight-threatening disease known as *Acanthamoeba* keratitis, which occurs mostly in contact lens wearers, and also can cause disseminating infections in immunocompromised individuals, which can result in granulomatous amoebic encephalitis (GAE)[4]. There is currently no compound available that are specific against free-living amoebas[5]. Research on development of anti-amoebic drugs has been in continuous activity for years, without significant breakthrough. Recently, redox networks in parasites were proposed as potential drug targets[6], a good example is trypanothione reductase in trypanosomatids[7]. For this reason, the drug-mediated inhibition of the large selenoprotein thioredoxin reductases (TrxR-L) of *Naegleria* and *Acanthamoeba* was studied here. In recent studies the FDA-approved gold-based compound auranofin (Ridaura®), which is able to inhibit thioredoxin and glutathione systems, was tested against *N. fowleri* and *Acanthamoeba* spp. showing promising results[8,9]. However, it was not tested if auranofin inhibited the amoeba TrxR-L enzymes, which was confirmed here. Moreover, the inhibition of TrxR-L with two new compounds named TRi-1 and TRi-2, which are specific against selenoprotein TrxR isoenzymes[10], and their *in vitro* activities against *N. gruberi* and *A. castellanii* in culture were investigated. The results suggest that TrxR-L can indeed serve as a potential drug target for therapies aimed at these amoeba species.

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P13

Molecular identification of potentially pathogenic free-living amoebae and their yeast endosymbionts isolated from oral cavity of COVID-19 patients in Iran

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Keywords: Free Living Amoebae, Endosymbionts, COVID19

Severe acute respiratory syndrome coronavirus-2 is a coronavirus that is responsible for the worldwide pandemic of coronavirus disease 2019. Free-living amoeba (FLA) have been reported from different clinical samples and organs. FLAs are believed to carry a couple of microorganisms as endosymbiont. This study aimed to investigate the presence of FLA in throat samples of COVID-19 patients admitted to hospitals and to study the presence of SARS-CoV-2 and selected yeasts in isolated FLA. Nasopharyngeal swabs (n= 60) were obtained from confirmed COVID-19 patients, and cultivated onto the 1.5% non-nutrient agar (NNA) plates. FLA was detected using morphological features and microscopic method. RNA and DNA extractions were performed to investigate the presence of SARS-CoV-2 and yeasts, respectively, using qReal-time PCR. The quality of RNA was checked by investigating *Acanthamoeba* sp., housekeeping gene, 18S ribosomal RNA. The genus, species, and genotypes of FLA were characterized using sequencing. From 60 Coivid-19 positive samples, 18 (30 %) were positive for FLA. PCR/sequencing results showed the presence of one or more FLA in a plate. In this regard, *Acanthamoeba* sp., *Naegleria australiensis*, *Tetramitus* sp., and *Vermamoeba vermiformis* were in 12 (60 %), 1 (4.54 %), 2 (9.1 %), and 7 (31.81 %) of PCR-positive samples. There was no statistical correlation between the presence of FLA and hospitalization in intensive care unit (ICU), vaccination history for COVID-19, and background diseases. qReal-time PCR was not positive for the presence of SARS-CoV-2 RNA in isolated FLA. Except three (16.67 %), all other samples (15; 83.33%) were positive for *Candida albicans* (11/18; 61.11%), *C. tropicalis* (3/18; 16.67%), and *C. parapsilosis* (3/18; 16.67%). In addition, *Geotrichum candidum* was detected among 10/18 (55.55%) of FLA samples. Our study is the first reporting the presence of FLA in clinical samples isolated from COVID-19 patients. Although trace of RS-CoV-2 RNA was not detected in isolated FLA, the presence of yeasts, particularly *C. albicans,* signifies the probable role FLA in transmission and initiation of secondary infections by fungi in susceptible patients.

P14

Evaluation of the effect of Mexican propolis against *Naegleria fowleri* and *Acanthamoeba* spp.

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Keywords: Free-living amoebae, propolis, flavonoids.

Free-living amoebae (FLA) are unicellular protozoa widely distributed in nature. Nevertheless, some species are able to induce several human and animal pathologies (1). *Acanthamoeba* and *Naegleria* are referred to as amphizoic organisms since exist as both FLA and pathogenic parasites. Till now, no agent has been described as an effective treatment (2). The investigation for potential and effective treatments against amoebae is gaining a wide interest in the public health research. Recently, the research was oriented into the natural products for finding alternative drugs (1, 2). Medicinal plants could be considered as a potential source for more efficient treatment since some of them have shown to be active against *Naegleria* and *Acanthamoeba* trophozoites (3, 4). The aim of the present work was to evaluate the anti-amoebic effect of Mexican propolis on *Naegleria fowleri* and *Acanthamoeba* spp. Mexican propolis extract was subjected to a gradient column chromatography (stationary phase: flash silica gel) with which 189 fractions were obtained, being analyzed with thin layer chromatography, from which the fractions were selected: F19, F35, F40, F51, F75, F80, F101, F130 and F160 and were analyzed by nuclear magnetic resonance. It was possible to identify compounds of phenolic origin such as pinocembrin, biochanin A, baicalein, pinobanksin chalcone, 5,8-dihydroxy-flavanone, and rhamnetin. Until now, the total extract of Mexican propolis had been analyzed; LC_{50} for *Naegleria fowleri* (ATCC 30808), *Acanthamoeba castellanii* (clinical isolate) and *Acanthamoeba culbertsoni* (ATCC 30171) was determined; 0.189 mg/mL, 1.212 mg/mL and 0.729 mg/mL respectively. Cell viability was determined by crystal violet technique. This is the first report on a Mexican propolis and its effect against FLAs, we consider that the anti-amoebic activity is directly related to the identified compounds since these have been reported with antimicrobial properties against various pathogens. In the present work we can conclude that extract has anti-amoebic activity on the three species studied, being *N. fowleri* the most susceptible. Besides, there are many compounds of phenolic origin in the propolis. At present we continue evaluating the effect of the compounds isolated from propolis against strains in study.

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P15

A multiplex qPCR-HRM assay for *Acanthamoeba* species genotyping

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Keywords: Acanthamoeba; qPCR-HRM; Genotyping

Acanthamoeba spp. are ubiquitous and opportunistic free-living amoebae (FLA) that are very common in nature and can cause amongst other conditions a severe eye infection called *Acanthamoeba* keratitis (AK). The diagnosis of *Acanthamoeba* infections has been facilitated and improved by the establishment of several PCR assays mostly targeting various regions of the nuclear small subunit 18S rRNA gene (Rns). The genus *Acanthamoeba* is classified into at least 23 genotypes (T1-T23) based on their 18S rRNA whole gene sequences. Studies have shown that not all genotypes are equally involved in eye infections, with genotype T4 being the most prevalent in the environment and commonly causing keratitis. We recently developed and validated a qPCR assay detecting and quantifying the most common genotype T4. In this study, we have established a cost-effective tandem multiplex-high resolution melting analysis to simultaneously detect and quantify those genotypes that have repeatedly been isolated from patients, including T3, T4, T5, T6, and T11. This assay will be a convenient and cost-effective method that could contribute to a quicker and accurate identification of *Acanthamoeba* species.

P16

A 4-Year retrospective study of the presence of thermophilic free-living amoebae in recreative geothermal baths in Guadeloupe.

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Keywords: thermophilic free-living amoebae; 18S metabarcoding; recreational water

Free-living amoebae (FLA) are ubiquitous protists found in soil and water. Some FLA such as Naegleria fowleri (NF), Acanthamoeba, Sappinia and Balamuthia can cause rare but fatal encephalitis [1]. In 2008, NF was responsible for the death of a 9-year-old boy who swam in a geothermal bath in Guadeloupe [2]. In 2013, our group showed that NF could be found in most of these baths [3], the soil being the origin of this contamination [4].

Herein, we analyzed for 4 years the diversity and abundance of thermophilic FLA in recreational geothermal waters in Guadeloupe, using metabarcoding and the most probable

number (MPN) method (for Naegleria sp). From 2018 to 2022, a total of 74 water samples were collected from 7 baths commonly used by Guadeloupean and tourists, and with different characteristics (natural, tiled, regularly cleaned or not, and with temperatures ranging from 27 to 40°C). DNA was extracted from FLA cultivated at 37-40°C to detect thermophilic FLA. Metabarcoding studies were conducted through FLA 18S rDNA amplicons sequencing [5]; amplicon sequence variants (ASV) were extracted from each sample and taxonomy assigned against PR2 database using dada2 and phyloseq tools. We also searched for Naegleria and NF using conventional PCR targeting ITS and NF-ITS [3] and we quantified these FLA using the MPN [6].

Our results showed that, over the 4 years, differences in FLA diversity and abundance were observed between the 7 baths, but without a clear seasonal distribution. Naegleria, Vermamoeba and Stenamoeba were the most represented genera, while the genera Acanthamoeba and Vahlkampfia were mainly found in 2 baths. The MPN values for Naegleria sp (NT/L) increased overall between 2018 and 2022 in almost all baths, but the MPN values for NF (NF /L) seem to decrease.

Globally, our results showed that, although we cannot establish a peak in FLA detection, the presence of FLA (namely Naegleria and Acanthamoeba) in recreational baths can pose a potential threat on human health in Guadeloupe. It is thus important to continue the regular control of these baths (requested by the local French health agency, ARS Guadeloupe), as a preventive health measure.

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P17

Comparative transcriptomic profiling of virulent and less-virulent isolates of *Naegleria fowleri* in mice brain: the amoeba and the host perspectives

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Keywords: Naegleria fowleri; mouse model; differential gene expression analysis

Naegleria fowleri (NF) is a free-living amoeba widespread in water and soil, that causes Primary Amebic Meningoencephalitis (PAM), a rare but one of the most devastating forms of necrotic meningoencephalitis in humans [1]. Innovation in methods of PAM treatment is required [2] but there is a limited understanding of the mechanisms of NF pathogenesis. It is also known that the degree of pathogenesis of NF isolates can vary [3–5], but the mechanisms underlying these phenomena and how this impacts amoeba-induced meningoencephalitis remains unclear.

Herein, we used two newly isolated NF environmental strains (NF1_MY and NF45_GC) with natural contrasting virulence phenotypes in C57Bl/6 mice brain. Mice were infected either with NF1_MY (less virulent) and NF45_GC (highly virulent) strains and total RNA was extracted from (i) the amoeba strains NF1_MY and NF45_GC isolated from brain samples, and (ii) NFinfected and uninfected mouse brain samples. These samples were then subjected to transcriptome analysis using RNA sequencing (RNA-seq) to identify differentially expressed genes (DEGs). Differential gene expression analysis identified a total of 874 and 915 DEGs ($|\log2\ Fc| > 2$, p-value < 0.01) in the virulent NF45_GC and less virulent NF1_MY strains (respectively), a set of 220 DEGs being shared by both strains. Functional enrichment using the Cytoscape StringApp showed that these DEGs are significantly related, but not limited, to metabolic processes, response to stress, regulation of cell cycle, posttranslational modification, and motility. Furthermore, our analyses identified a total of 3623 and 438 DEGs ($|\log2\ Fc| > 2$, p-value < 0.01) in mouse brains infected by NF1_MY and NF45_GC, respectively.

Functional enrichment revealed that these DEGs induce major changes in host cell immune responses, regulation of cellular processes and regulation of cytokine production, highlighting a dynamic amoebae-host interaction in particular for the less virulent NF1_MY strain.

Combined, the results from these analyses allow to elucidate potential determinants of virulence and pave the way for future PAM research and the development of novel therapeutics.

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P18

Updating the *Acanthamoeba* DNA databases to 2023

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Keywords: Ribosomal RNA genes, DNA Databases, Sequence Types

Amoebae in the genus Acanthamoeba were discovered by Castellani and described by Volkonsky in 1930. Morphological characters (primarily cyst and trophozoite structure), and cytological characteristics of nuclear division were used to describe different species (Pussard and Pons, 1977). Uncertainty regarding morphological approaches led to the hope that molecular methods would provide more definitive subgeneric classifications. Comparison of isolates focused on the nuclear small subunit ribosomal RNA gene (Rns), with sequences deposited in the International DNA databases (GenBank, DDBJ and EMBL). Accumulation of information for the Rns gene led to the development by our lab of the Sequence Type classification, now routinely used for classifying isolates of Acanthameoba. Since 1996, when 4 sequence types were identified, additional reports elevated the number of types to 23. In 2022, data on full or partial Rns sequences exist for ~6300 isolates of Acanthamoeba, an increase of ~1200 sequences since FLAM2019. Information from "almost complete" Rns sequences (sequences > 2000 bases) exist for 813 isolates, with 524 sequences identified as T4 isolates (64%), a percentage slightly lower than that for isolates with only partial Rns sequences (70%). T4 is easily the most common Sequence Type in either environmental or clinical samples. The only other Sequence Type exceeding 5% is T5 (7.0%). No Sequence Type identified post-1998 represents more than 0.65% of isolates, except Type T15, associated with A. jacobsi (2.6%). Several Sequence Types contain significant phylogenetic subgroups (sequence divergence between 1-5%). At least seven significant sub-types exist within T4, and five sub-types exist within the boundaries of the super-Type T2-T6. Current species nomenclature within Acanthamoeba is only loosely related to phylogenetic classification. Complete mitochondrial genome sequences exist for 33 isolates. Other major portions of the DNA databases include information on several mitochondrial loci [~210 mitochondrial rns sequences, 142 sequences for cytochrome oxidase subunit I, and 67 sequences for NADH dehydrogenase subunit 5 (ND5)]. Multi-isolate information exists for a small number of nuclear proteins, including 50 sequences of beta-tubulin, 65 sequence of elongation factor-1, 55 sequences of glyceraldehyde-3-phosphate dehydrogenase, and over 30 sequences of three other genes. Information on all sequences, including much additional analysis is summarized at our website: http://http://u.osu.edu/acanthamoeba/.

P19

Utilization of Mass Spectrometry for the Detection of *Naegleria fowleri*

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Keywords: Naegleria fowleri, Ergosterol, Mass Spectrometry

Naegleria fowleri, the causative agent of Primary Amebic Meningoencephalitis (PAM), presents in patients with nonspecific symptoms which include stiff neck, vomiting, fever, chills, light sensitivity, headache, and seizures. These nonspecific symptoms often lead to an initial misdiagnosis of bacterial meningitis in patients, delaying the appropriate treatment. Due to these challenges, an ideal method of detection in cerebrospinal fluid (CSF) would target a component of N. fowleri that is not present in bacterial or human cells. A method for detecting ergosterol from Naegleria fowleri in cell culture media was developed using liquid chromatography-mass spectrometry, with hexadeuterated cholesterol as an internal standard. Initial experiments showed that ergosterol could be efficiently extracted (95-110%) from conditioned cell culture media with acetonitrile. Calibrators were prepared by fortifying cell culture media with different concentrations of ergosterol. We observed a linear relationship between peak area ratio and ergosterol concentration from 5 to 1,260 nmole/L . To investigate the ability to detect Naegleria fowleri in cell culture, samples were removed from an actively growing culture on four consecutive days following the transfer of ~$5x10^6$ amoeba to ~25 mL of fresh media. The concentration of ergosterol in the culture supernate increased from 124 nmole/L on day 0, to 224, 345, 406, and 585 nmole/L on days 1-4 respectively. Overall, the findings proved that it is possible to develop a quantitative method for analyzing concentrations of ergosterol present in cell culture fluid from N. fowleri. Following a full analytical validation of the method, we will adapt it to testing human CSF specimens.

P20

Genotyping of clinical cases of *Acanthamoeba* Keratitis from Indian patients

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Keywords: Acanthamoeba Keratitis; genotyping; Acanthamoeba lenticulata.

Over the years the number of patients with *Acanthamoeba* keratitis (AK) has increased in the geographic location of India. Most of these clinical cases were not contact lens wear related but caused by trauma or farming activities. Beside this, the keratitis cases were associated to fungal co-infections with *Acanthamoeba* [1,2]. In this study a collection of over 78 *Acanthamoeba* ocular isolates from AK cases as well as from fungal co-infections were genotyped for diagnosis and identification of several species of *Acanthamoeba* spp.

All isolated *Acanthamoeba* were cultivated in non-nutrient agar (NNA) directly from all stromal keratitis cases and were incubated at 26 ºC. Then, in order to extract DNA from monoxenic cultures, amoebic culture suspensions were directly placed into a Maxwell® DNA purification kit sample cartridge. PCR amplification of the 18S RNA gene from extracted DNA, was carried out using universal primer for *Acanthamoeba* genus.

In this study, 7 different species belonging to 6 distinct genotypes of *Acanthamoeba* from 78 positive samples were obtained. Genotype T4 was de the most frequent, whereas the most common species was *Acanthamoeba lenticulata.*

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P21

Isolation of *Acanthamoeba* sp. from the Tajogaite volcano, La Palma, Canary Islands

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Keywords: Acanthamoeba; La Palma; volcano;

Free-living amoebae (FLA) are a group of ubiquitous protozoa that have managed to be isolated from a multitude of samples, such as soil, air, or water. *Acanthamoeba* spp., *S. pedata*, *Paravahlkampfia* spp., *Vahlkampfia* spp., *N. fowleri*, *B. mandrillaris*, and *Vermamoeba* spp. have previously been described as opportunistic pathogens, triggering severe pathologies such as keratitis, encephalitis, or skin disorders. In addition, FLA can act as a vehicle for other pathogens, such as viruses or bacteria.

Among the FLA, the pathogenic amoeba that has been most successfully isolated is Acanthamoeba spp. Previous studies have isolated *Acanthamoeba* spp. in a multitude of environments, including extreme conditions, such as, high salinity seawater, high temperatures in snow or in mountains at an altitude of over 4000 meters.

The Tajogaite volcano is in La Palma Island, in the Canary Islands, Spain. The eruption of this volcano began on 19th September 2021, culminating on 13rd December of the same year, after 85 days of eruption, making it the third longest eruption in the history of the archipelago. From a sample of volcanic rocks, collected 6 months after the end of the eruption of Tajogaite volcano, 0.5 grams were sown on a 2% non-nutritive agar plate. This plate was incubated at room temperature and monitored daily until visualization of FLA. After obtaining an axenic culture of amoebae, DNA extraction was performed. Finally, PCR was performed using primers JDP-1 and JDP-2 and subsequent sequencing of the DF-3 region of *Acanthamoeba* 18S rDNA. In addition, a study was carried out to observe thermotolerance and osmotolerance, incubating at different temperatures and mannitol concentrations. *Acanthamoeba* sp. genotype T4, the most common genotype in soil isolation, was isolated from the sample studied. Furthermore, this amoeba has better grown in low temperatures and low salt concentrations (28°C and 0.1 M mannitol).

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P22

Interlaboratory comparison of methods for the detection of *Acanthamoeba* added to artificial corneal scraping samples or DNA

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Keywords: Acanthamoeba, PCR, diagnosis

To investigate the accuracy of methods (PCR and culture) for the detection of *Acanthamoeba* in clinical specimens, artificial corneal scrapings to which known amounts of *Acanthamoeba* cells had been added were sent to 24 French Centres. 22 laboratories were able to perform the detection of *Acanthamoeba*. 10 laboratories did PCR and culture, 8 only culture, and 4 only PCR. Different protocols of PCR were used with at least 7 methods of DNA extraction and 9 PCR assays. Different protocols of culture were also used in solid or liquid mediums.

The PCR compliance rate was 78.6% (83.3% for real-time PCR and 66.6% for conventional PCR). The culture compliance rate was 86%.

These findings highlighted a need for optimization of protocols of extraction / PCR as culture in some laboratories. Furthermore, the results confirm the need for an external quality assurance scheme to support laboratories involved in the diagnosis of *Acanthamoeba* keratitis.

P23

Investigating the relationship between *Neoparamoeba perurans* and *Vibrio spp*. and its influence in Amoebic Gill Disease.

Emma O'Halloran

Amoebic gill disease (AGD) is caused by *Neoparamoeba perurans* (*N. perurans*). The disease has devastating effects on the global aquaculture industry and farmed teleosts, such as Atlantic Salmon are highly susceptible to this disease. *N. perurans* are free-living in the marine environment and infect the gills of teleosts under specific conditions, for example, high salinities and high temperatures are believed to influence outbreak occurrences. With this in mind, deeper knowledge of this opportunistic pathogen is required to understand infection routes and transmission, effective treatments and to improve mitigation strategies, especially with the rise of sea temperatures as a result of climate change.

The gills of fish are abundant in different microorganisms, especially bacteria and we hypothesise that this protist may depend on certain bacterial species for its survival and/or virulence capabilities. Importantly, we demonstrated through Fluorescence in situ hybridisation (FISH) that *Vibrio* species were predominantly intracellular in a culture of *N. perurans* retrieved from the west coast of Scotland. To investigate the relationship between *N. perurans* and *Vibrio* species further, a survey of gill swabs from Salmon farms in Scotland were tested for *N. perurans* and *Vibrio* presence through in vitro culture and molecular techniques. This data will be used to investigate the relationship between *Vibrio* species and AGD prevalence and disease severity. This will provide insight into how intra-amoebal bacteria may influence amoebic disease outcomes.

P24

Identification of surface immunogens from *Balamuthia mandrillaris*

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Keywords: Balamuthia; proteomics; biomarkers.

Balamuthia mandrillaris is a free-living ameba responsible for causing a rare disease named granulomatous amoebic encephalitis (GAE) and infections in other organ systems including the skin. GAE is usually a fatal infection of the central nervous system that can affect immunocompromised and immunocompetent individuals with a mortality rate more than 95%. Currently, there are approximately 200 cases reported worldwide, which 96 cases correspond to America. The incubation period is unclear, but its course is subacute to chronic and usually ends in dead because the diagnosis of the disease is commonly post mortem since there is not recognized globally and it exists a lack of a proper diagnosis and an effective treatment. In this way, the main goal of this work was to identify surface proteins of *B. mandrillaris* to elucidate possible biomarkers that can be used as a tool for an opportunistic diagnosis and/or treatment. For this reason, we worked with axenic cultures of *B. mandrillaris* using BMI medium supplemented with 10% bovine serum at 37°C that were harvested to obtain the total protein extract as well as the membrane and cytosolic proteins using a Kit. The samples were analyzed by SDS-polyacrylamide and two-dimensional (2-D) gel electrophoresis. Some gels were electroblotted onto a nitrocellulose membrane for Western Blotting where anti-*B. mandrillaris* serum from an immunized rabbit was used. Besides, a shotgun analysis was applied to have a more complete profile of this microorganism. As a results, we found in the 2-D gel that *B. mandrillaris* has a protein profile from approximately 11 to 185 kDa that when was compared with the Western Blot, we could observe spots ranging from 14 to 135 kDa, but with more prevalence in the range of 30 to 80 kDa. Analyzing the blots, we have established five possible candidates corresponding to approximately 35, 42, 45, 85 and 100 kDa to identify by mass spectrometer and compare with the shotgun analysis to carry out another study that allow us to elucidate the function of these proteins and if they are implicate in the microorganism's adherence.

P25

High throughput cross kingdom diversity analysis in multiple environments

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Keywords: High throughput sequencing; Biodiversity; Microbial interactions

Protists, and free-living amoebae (FLA) in particular, can be found in numerous environments, and particularly water (fresh and marine) but also soils. To assess their presence and diversity, cultured-based methods are often used even though they could miss a large portion of these organisms. As an alternative, sequencing of ribosomal RNA genes can provide a better understanding and a larger view of the protist diversity, despite inherent limits.

Nonetheless, even with widespread use of high throughput sequencing, the diversity of FLA is rarely investigated in environmental samples - especially in soils - even if they may represent a large fraction of their diversity (Geisen et al., 2017). Thus, our understanding of FLA community composition and dynamics remain to this day very scarce. Added to that, despite the facts that multiples protists, FLA included, use phagocytosis to feed, very few studies document the diversity and abundance of bacteria associated with them.

Therefore, the aim of this work is to precisely analyze, through high throughput deep sequencing, the presence and the relative abundance of protists, with a focus of FLA, and associated bacteria in various environments.

A selection of 15 different geographical sites that include ponds, lakes, river sediments, marine water and forest soil, were sampled, following seasons, for one year. During sampling, abiotic parameters such as temperature, dissolved oxygen, oxidoreduction potential, salinity and conductivity were recorded. From those samples, total DNA was extracted and served as a matrix for 16S and 18S ribosomal RNA gene sequencing, using Illumina and Oxford Nanopore technologies, respectively.

The sequence analyses will allow to precisely document FLA communities and their dynamics, possibly in link with abiotic parameters. The confrontation of protists and bacterial diversity will allow to formulate hypotheses regarding putative FLA associations with bacteria.

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P26

Naegleria fowleri in Central and South America: The brain-eating amoeba is it in Perú?

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Free-living amoebae (FLA) are microbial entities that are capable of causing disease in humans and animals. Naegleria fowleri is one of the three free-living amoebae that are pathogenic to humans; it has been identified in the South American continent. Peru has reported only one case in 2015. This case has not been published, only notified to the Ministry of Health. The girl under 11 years old and was infected in Piura, north coast of Peru. Patients die after 5-7 days, that is, it is an acute course. Latin countries such as Argentina, Brazil, Venezuela and Colombia describe 1, 5, 7 and 6 cases respectively. In this review, we will describe various aspects of this amoeba. Morphology, ecology, pathology, epidemiology, classical histological pattern, adequate culture media, and microbiological and molecular diagnoses. In addition, the relationship with the phenomenon “El Niño” and the appearance of cases of meningitis.

P27

Morphological and molecular analysis of *Acanthamoeba* and other free-living amoebae in soil attached to *Solanum tuberosum* (potato) sold by farmers in a market in northern Lima - Peru

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Backgrounds: In Peru and other Latin-American countries, potatoes are usually sold as harvested, that is, the soil on the potatoes is not removed and, in this status, they arrive to the main markets of capitals. In Lima, tubers as potatoes are sold with soil stick on them. It is well known that the soil contains many microorganisms that can be potentially pathogenic, one of them being free-living amoebae. Objective: To determine the presence of ***Acanthamoeba*** and other free-living soil-attached amoebae on *Solanum tuberosum* sold by farmers in a market in Lima-Northern Peru. **Material and Methods**: The study was a cross-sectional descriptive observational study. Five huayro potatoes purchased at a market in northern Lima were evaluated. In the laboratory, a saline solution for amoebae (sol. Page) was used to obtain the soil attached to the potatoes (this attached soil is the main sample for the investigation). The soil was concentrated in sterile wide-mouthed jars and finally seeded on non-nutrient agar plates with *Escherichia coli* and without *Escherichia coli*. **Results**: Five strains of *Acanthamoeba* genotype T4, and one strain of *Darbyshirella* were identified by PCR. In addition, other amoebae such as *Leptomyxa*, *Arboramoeba*, and *Angulamoeba* were morphologically identified. **Conclusions**: Free-living amoebae never before described in Peru were identified, these grew only on non-nutrient agar plates without any food source, with a growth time of 4 to 6 weeks in soil attached to potatoes from a market in Lima-North. In addition, potentially pathogenic amoebas of the genus *Acanthamoeba* genotype T4 were identified with a live *Escherichia coli* as a nutritional source.

P28

Isolation of *Acanthamoeba sp. y Balamuthia mandrillaris* from commercial soil from a supermarket in Lima

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Background: The high lethality rates in cases of human infections caused by free-living amoebae and their role as pathogenic microorganisms prompted their identification in environments such as soil. Currently, inputs are used to prepare substrates of ornamental plants in gardening, being farmland soil one of the most used because it can be mixed with other materials called ''soil mixtures''. This commercial soil is distributed in our country. **Objective**: To isolate and identify *Acanthamoeba spp*. and *Balamuthia mandrillaris* from samples of commercial soil from a supermarket in Lima obtained in August 2019. Study design: A crosssectional descriptive observational study was conducted. **Material and methods**: 30 bags with commercial soil were used, each soil sample from bags was sowed on Non-Nutrient Agar (ANN) enriched with *Escherichia coli* ATCC 25922, the identification of free-living amoebae was performed by PCR of the positive isolates. Results: Acanthamoeba spp. and one suggestive amoeba of the genus Vahlkampfia were isolated from 66.7% (20/30) and 3.3% (1/30) of commercial soil samples, respectively. *Balamuthia mandrillaris* was not isolated. **Conclusions**: The presence of potentially pathogenic amoebae in commercial soil distributed in a supermarket of Lima was evidenced, being the amoebae genus Acanthamoeba the most frequent.

P29

Gut bacteria of water monitor lizard are a potential source of antiamoebic molecule(s)

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Water monitor lizards (WML) thrive in unsanitary conditions, feed on rotten meat, are exposed to heavy metals and are among the very few species to endure the catastrophic Cretaceous-Tertiary extinction event. We speculate that their microbial gut flora may produce substances contributing to their "hardiness". In this study, we characterized the gastrointestinal tract of WML using 16S rDNA sequencing and prepared conditioned media of the selected gut bacteria. Amoebicidal assays revealed that conditioned media exhibited antiamoebic effects against *A. castellanii*. Conditioned media inhibited encystation and excystation of *A. castellanii*. In addition, conditioned media inhibited adhesion of amoebae to human cells. Finally, conditioned media showed limited cytotoxic effects but reduced amoebae-mediated host cell death. LC-TIMS-QTOF MS, revealed the identity of these gut microbial metabolites. The results revealed a total of 189 identified metabolites. Among the identified metabolites, the most abundant compounds were (+)-(S)-Carvone, 1,11-Undecanedicarboxylic acid, 1,3,5-Trimethoxybenzene, 3-Methylindole, 4-Ethylphenol, 4-Guanidinobutanoic acid, 5-Hydroxytryptophol, 6-Dimethylaminopurine, Acetaminophen, Acrylamide, Benzoic acid, Biotin, Doxylamine, Hydrocinnamic acid, Indole-3-carbinol, Indolelactic acid, L-Acetylcarnitine, Quinaldic acid, Succinic acid and several other compounds. Overall, mass spectrometry revealed several antimicrobials, anticancer, neurotransmitters, anti-depressant and other metabolites with biological functions. Overall, these findings imply that gut bacteria of WML produce molecules with anti-Acanthamoebic capabilities, which could eventually lead to the development of therapeutic antiamoebic medications, however further research is needed to realize these expectations.

P30

Ultraviolet – Chlorine combined treatment efficiency to eliminate Naegleria fowleri in artificial surf lagoons

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Keywords: Naegleria fowleri, UV light-Chlorine combination, Artificial Surf Lagoon

Naegleria fowleri, a protozoan belonging to the free-living amoeba group, is the causative agent of Primary Amoebic Encephalitis (PAM). PAM is a central nervous system disease that is fatal in more than 95% of the reported cases. This parasite can be found in warm water bodies such as lakes, rivers, or inadequately disinfected swimming pools. On the other hand, chlorination and UV light treatment are two of the most extensively used disinfection methods in recreational water facilities. In the present study, the effect of chlorination and UV light on N. fowleri trophozoites was studied in a closed water circuit with the aim to assess the efficacy of these disinfection methods in large pools. The obtained results showed that the chlorination was able to decrease the number of viable cells despite the elimination was not totally achieved. Nonetheless, the combination of the UV light with the chlorination allowed the complete removal of the N. fowleri trophozoites from the water in experimental testing conditions.

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P31

Differential gene expression in *Acanthamoeba spp.* in response to contact lens materials

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Keywords: Acanthamoeba, keratitis, gene expression

Acanthamoeba keratitis is a serious corneal infection which is extremely difficult to treat and can often lead to blindness. This disease has become increasingly more prevalent and important to patient safety with the steady increase in the use of contact lenses. *Acanthamoeba* keratitis is most often associated with contact lens usage and contaminated water contact. Previously, the only confirmed role of contact lenses in *Acanthamoeba* keratitis infection was as a mode of transmission to the eye. We hypothesized that *Acanthamoeba* genomic profile and behavior on contact lenses depends on material and that behavior may have an impact on the transmissibility to the ocular surface of humans.

Following the observation of material-dependent *Acanthamoeba* behavior leading to either independently motile trophozoites or encysting spheroids, we analyzed the transcriptome of *Acanthamoeba* ATCC 30461 on three contact lens materials and a no lens control. This study was undertaken to identify which genes or potential pathways may be contributing to the trophozoite aggregation and downstream biological changes in cells as a result of being part of a spheroid.

744 genes were significantly differentially expressed at 4, 12 and 24 hour in lehfilcon A (non-aggregating lens) vs. comfilcon A and samfilcon A (aggregating lenses). The genes were analyzed for homologous genes from the Neff genome and potential functions identified. Protein-protein interactions and the significantly differentially expressed pathways were further identified and significant common pathways were identified using STRING. Gene expression changes between lens materials had pathways involving the actin cytoskeleton, intracellular vesicle formation, autophagy and metabolic activities. These genomic alterations matched the visual results of *Acanthamoeba* on different materials.

In conclusion, we show that significant gene expression changes occur in response to *Acanthamoeba's* interaction with contact lenses, which coincided with material-dependent aggregation and encystment, and a significant resistance to disinfection. Previously, amoeba on aggregating lenses were found to be more difficult to disinfect using common commercial contact lens multipurpose solutions than they were on non-aggregating lenses. These alterations may impact patient safety and contact lens materials should be considered in the conversation of reducing ocular infections.

P32

Glutathione S-Transferases play an important role for viable cyst production in *Acanthamoeba*

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Keywords: Glutathione S-transferase, Acanthamoeba, Encystment

Acanthamoeba is a cosmopolitan protozoa capable of causing a corneal infection known as *Acanthamoeba* keratitis (AK). They have a biphasic lifestyle composed of a vegetative trophozoite and a latent cyst. The cysts are particularly problematic as they facilitate the persistence of AK. Therefore, blocking encystment might be the key to deal with encysting protozoan infections. The objective of this research was to identify genetic factors involved in *Acanthamoeba* encystment using mRNA sequencing. We have identified Glutathione S-Transferases (GST) as factors vital for cyst viability and survival in *Acanthamoeba*. Four different timepoints were selected for sequencing during encystment: 0 hours, 24, 48 and 72. Differential expression analysis was performed from the sequencing results. Upregulated genes were compared with amoebaDB to identify their function. Hypothetical proteins were then searched using BLAST to identify similar or related proteins. Five upregulated hypothetical proteins showed a strong similarity to a Glutathione S-transferase (GST) domain. Encysting cultures were treated with GST inhibitors (such as ethacrynic acid, and sulfasalazine) and cyst viability was measured using trypan blue. Ethacrynic acid showed that more than 60% of the cysts were not viable. Meanwhile, sulfasalazine showed a decrease of 40% on cyst viability. This indicates that GSTs are involved in the encystment process of *Acanthamoeba*. In several other protozoans, GSTs play a role in cell cycle and regulating the redox state, which we show are important to keep cell viability during encystment in *Acanthamoeba*. GSTs play an important role balancing the redox state and detoxification during encystment through all the metabolic changes happening allowing it to remain viable. Cysts are the main reason for AK persistence therefore one of the main challenges in treating the infection. Supplementing normal treatments using GST inhibitors, or targeting GST should decrease the probability of relapse for AK. Moreover, this could help prevent infections from potentially pathogenic bacteria hosted by *Acanthamoeba*.

P33

Acanthamoeba and *Legionella* in the hospital environment

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Free-Living Amoeba (FLA) are protozoa that are ubiquitously found in the environment and can act as human pathogens. One of these FLA, Acanthamoeba, is also considered the Trojan horse of nature as they can harbor other pathogens. This is the case of Legionella, a bacterium that causes respiratory diseases by inhalation of contaminated water aerosols. The most dangerous species for humans is L. pneumophila, although others have been described, such as L. feeleii, capable of causing pulmonary and extrapulmonary diseases.

This study studied the presence of these pathogens in the hospital water system to determine their role in acquiring nosocomial diseases. Water samples were obtained from three Spanish hospitals: two Madrid hospitals, H1(119 samples from different areas) and H2 (102 samples from various pediatric areas), and one Sevilla Hospital (86 samples from the water hemodialysis station).

A Real-Time Triplex PCR for detecting FLA (Acanthamoeba, Balamuthia mandrillaris and Naegleria fowleri) and two PCRs for detecting Legionella were performed, one capable of detecting the 5S subunit gene and the other for detecting the MIP gene, allowing to differentiate between species.

Acanthamoeba was detected in several samples, while the absence of Balamuthia mandrillaris and Naegleria fowleri species were not detected. The results of Acanthamoeba in the hospitals were: 64.71%-H1, 60%-H2, and 74,70%-H3. It is essential to highlight the 100% positive samples from the area of pneumology (H1) and the general pediatric area (H2). In H3, the highest positive percentages were 90% of the raw water samples and 75% at the circuit's final. These results suggest that Acanthamoeba survives the water circuit or colonizes the whole system.

Legionella sp was detected in the studied water circuit (18.33%-H1; 45.10%-H2; 39,53%-H3), with the presence of both L. feeleii and L. pneumophila.

These results show the need to assess the presence of Acanthamoeba in the intrahospital environment as these amoebae can favour nosocomial infections protecting bacteria, such as Legionella, from harsh environmental conditions such as biocidal reagents.

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Novel properties of deep eutectic solvents against *Acanthamoeba castellanii* belonging to the T4 genotype

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This aim of this study was to assess the anti-parasitic properties of deep eutectic solvents against clinical isolate of *Acanthamoeba castellanii* belonging to the T4 genotype. Assays were performed to investigate the effects of various deep eutectic solvents on *Acanthamoeba castellanii,* comprising amoebicidal assays, encystment assays, excystment assays, cytotoxicity assays by measuring lactate dehydrogenase release in human cells, and cytopathogenicity assays to determine parasite-mediated host cell death. In a 2h incubation period, deep eutectic solvents exhibited up to 85% amoebicidal activity at micromolar doses, which was enhanced further following 24 h incubation. When tested in encystment assays, selected deep eutectic solvents abolished cyst formation and were able to block excystment of *A. castellanii*. All solvents exhibited minimal human cell cytotoxicity. Finally, the majority of deep eutectic solvents inhibited amoeba-mediated cytopathogenicity. Deep eutectic solvents show potent antiamoebic effects. These results are very promising and to the best of our knowledge, are reported for the first time on the effects of deep eutectic solvents on amoebae. These results can be applied in the development of new formulations of novel contact lens disinfectants against *Acanthamoeba castellanii.*

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Drug discovery for *Naegleria fowleri*: a critical appraisal and target candidate profile to guide future studies

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Naegleria fowleri, causes an acute, fatal disease called primary amoebic meningoencephalitis (PAM). Although PAM is a rare disease, it results in >97% mortality rate, which ranks as one of the most virulent infectious diseases of humankind. A major unmet medical need is more effective, rapidly acting therapeutics. Drugs that are currently used to treat PAM and other pathogenic free-living amoebae have not been discovered and developed prospectively. Instead, Amphotericin B, antifungals, antibiotics and microbiocides available for other indications are used in a cocktail of drugs in an empirical approach to treat these infections. These drugs often are found to possess limited to moderate efficacy in vitro or in the mouse model of PAM. Clearly new approaches and new drugs are urgently required. Despite renewed interest in discovery of new drugs for PAM, the current approaches are confounded by several problems. Perhaps the most important is the definition of potency; there are too many studies that report 'potential drugs' with EC50s in vitro >20 uM, a range that is well outside the clinically achievable therapeutic range for most drugs. Secondarily, pharmacokinetic and pharmacodynamic studies of anti-Naegleria drugs are severely lacking and given the lack of potency observed, the bioavailability of proposed drugs at such high required exposure levels are unlikely to be obtained. Third, miltefosine is putatively called a 'wonder drug', yet it is weakly potent in vitro and in the mouse model. Finally, experimental evidence and rationale are lacking for why some drugs are included in the current treatment cocktail. Herein, I propose a target candidate profile (TCP) to guide drug discovery for PAM. The important criteria include rate of onset of action, potency against multiple strains, mechanism of action, physiochemical properties, blood-brain barrier permeability, drug-drug interactions, drug combinations, safety, regulatory approval, pharmacokinetics, and target identification. The proposed TCP outlines 'minimal essential' and 'ideal' criteria with the goal of guiding future drug discovery efforts and helping prioritize among the various chemical scaffolds that have been reported as drug candidates in the literature. I acknowledge support from US NIH (R03AI141709) and the Georgia Research Alliance.

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Evaluation of the role of naegleriapores A and B during the early stages of primary amoebic meningoencephalitis in mouse model

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Key words: meningoencephalitis, naegleriapores, virulence factors

Naegleria fowleri, the causative agent of primary amebic (PAM), possesses a large number of virulence factors involved in the development of the disease. Microbial virulence factors encompass a wide range of molecules produced by pathogenic microorganisms that enhance their ability to evade host defenses and cause disease. This broad definition includes secreted products such as toxins, enzymes, exopolysaccharides, as well as cell surface structures such as capsules, lipopolysaccharides, glycoproteins and lipoproteins. Therefore, virulence factors are a microbial component that promotes growth or survival during infection. In this work, we evaluated the role of naegleriapore A and B in the pathogenesis of the disease in an in vivo mouse model. To evaluate their role in the early stages of MAP, 20aa immunogenic peptides were designed using bioinformatics strategies from naegleriapore protein sequences reported in biological databases. Once designed and synthesized, they were used to produce polyclonal antibodies in rabbits. At the end of this scheme, the purified serum was used for immunoassays such as Wester-blot, cytochemistry and histochemistry. In particular, naegleriopore A localization was observed at 8, 12 and 24 h post-infection. At 8 h, the amoeba was localized in the lumen of the nasal cavity, on the apical side of the respiratory epithelium and in the lamina propria. The distribution of naegleriopore A was on the trophozoite membrane. At 12 h, it was already observed that the amoeba penetrated the olfactory epithelium, reaching the olfactory bulb, where the naegleriopore distribution was visualized with greater intensity. Finally, at 24 h, the amoeba located mainly in the olfactory bulb and frontal lobe of the brain expressed naegleriopore A in an intense and more regionalized manner towards the food vessel structures. To evaluate the involvement of these molecules during the pathogenesis of disease caused by N. fowleri, naegleriopores were blocked by incubating trophozoites with anti-Naegleriopore A and B IgG antibodies, followed by a survival assay in which up to 40% survival was obtained.

These results suggest that these glycoproteins play an important role in amoeba pathogenesis.

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Bioinformatics design of a peptide from the Naegleria fowleri membrane protein MP2CL5 conjugated with a non-toxic modified adjuvant from the native Cholera Toxin A1 structure that can be used in the protection model of meningitis in mice

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Naegleria fowleri is the etiological agent of Primary Amebic Meningoencephalitis considered one of the most acute and fatal diseases that exist for humans. It has been reported that, despite the therapeutic options available against the disease, only 5% of the population survives the infection. Therefore, the need arises to have vaccines that confer protection against this disease and in addition to developing adjuvants to enhance the immune response. In this regard, in our work group we obtained a peptide designed from the membrane protein MP2CL5 of N. fowleri (Smp145) that was shown to be immunogenic; however, it would be of great importance to enhance its immunological response, being able to co-administer it with a non-toxic adjuvant. Therefore, the objective of this work was to carry out the bioinformatic design of a peptide of the *N. fowleri* membrane protein MP2CL5 conjugated with a non-toxic modified adjuvant from the native A1 structure of cholera toxin (CTA1). For which different bioinformatics tools were used to obtain a model with a modification in amino acid 61 of the CTA1, to which the Smp145 peptide was added and both molecules were joined with a 13-glycine linker. As for the results obtained, the mutation in CTA1 bound to the peptide, produces a reduction in the toxicity of the molecule in silico experiments, likewise, the prediction in the binding of Smp145 to the receptor of B cells (BCR), suggests that the molecule is directed specifically to the BCR, decreasing its native enzymatic activity. The stereochemical evaluation showed that the generated model has a high number of adequately predicted residues. In the ERRAT test, the confidence with which it is possible to reject regions that exceed the error values was evaluated, in the generated model a high score was obtained, which determines that the model has a good structural resolution. Therefore, the design of the conjugated peptide in this work will allow us to proceed with its chemical synthesis and subsequently be able to use it in the mouse meningitis protection model caused by N. fowleri.

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Memory Immune Response in Mice against *Naegleria fowleri* infection

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Key words: *Naegleria fowleri*, memory response, immunization

Vaccines work by eliciting an immune response and consequent immunological memory that mediates protection against infection disease. This response can generate different mechanisms of the adaptive immune system, such as CD4 T cells, CD8 T cells, B cells, and long-lasting antibody responses. The understanding of the immunological memory in our immunization model against the primary amoebic meningoencephalitis (PAM) in mice may contribute to the development of efficacious and convenient vaccines against *N. fowleri* infection. Therefore, we analysed if four intranasal immunizations with *N. fowlery* lysates plus cholera toxin (CT) as adjuvant contribute to the memory response. After 3, 6 and 12 months of the last immunization with or without booster, populations of T and B lymphocytes from nose-associated lymphoid tissue (NALT), cervical lymph nodes (CN) and nasal passages (NP) were analyzed by flow cytometry, as well as IgA and IgG antibodies level from serum and nasal washes. The percentage of memory CD4 T cells were increased mainly in NP either with or without booster. Whereas memory B cells from NALT of mice with booster immunization expressed the highest levels of CD80, CD19 and CD27 after 3 months of the last immunization followed by cells from NP and CN. *N. fowleri*-specific IgA and IgG antibodies production also persisted after 3 and 6 months. This memory immune response might determine the survival rate found (60%) against *N. fowleri* infection. These results suggest that our immunization scheme induces memory response, which is essential for future vaccine development.

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Effect of target tissue on erythrophagocytosis of amphizoic amoebae

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Keywords: erythrophagocytosis, Acanthamoeba, Naegleria

Amphizoic amoebae are protozoa capable of living as free-living organisms and as opportunistic pathogens. *Naegleria fowleri* and *Acanthamoeba* spp. stand out for their clinical importance since they are etiological agents of infections in the central nervous system with high mortality for which there is no effective treatment.

The aim of this work was to determine if the target tissue induces and/or exacerbates the process of erythrophagocytosis in *Acanthamoeba* spp. and *Naegleria* spp.

The study was carried out with two different target tissues; fibronectin obtained from human blood and cerebral mouse tissue; extracts were obtained and distributed in the form of micropatterns (1), to facilitate the interaction with 1.5×10^5 *A. castellanii, A. culbertsoni, N. fowleri* and *N. lovaniensis* trophozoites/1ml of culture medium and incubated for 30 min at the optimal temperature of each strain, posteriorly, erythrocytes were added in a ratio 1:4. Video recordings of 500 trophozoites were performed in each assay, in which the number of phagocytosis events, the number of phagocytosed erythrocytes were evaluated, as well as the emission of phagocytic structures and trogocytosis.

Both the analysis of the control and experimental groups showed that the phagocytosis process was less than the trogocytosis process. Fibronectin induced a slight increase in these events. Likewise, it is suggested that the trophozoites under study; *Acanthamoeba* spp. and *Naegleria* spp. have the ability to phagocyte and to trogocyte; with any stimulus, emitting different types of phagocytic structures. No previous contact is necessary for this process to take place or be exacerbated.

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