XVIITH FREE-LIVING AMOEBAE MEETING

Zarzis , Tunisia, 11th-15th April 2017

On behalf of the Laboratory of Materials Molecules and Applications of the University of Carthage it is our pleasure to welcome you to the 17th International Meeting on the Biology and Pathogenicity of Free-living Amoebae held in Djerba- Zarzis between 11th and 15th of April 2017

We would like to express our gratitude to our honorable partners, sponsors and to all the people who contributed to organize this meeting:

FLAM 2017 SCIENTIFIC PROGRAMME

TUESDAY 11 APRIL 2017

16:00-19:00: Registration
19:30- 21.30: Welcome Party

WEDNESDAY 12 APRIL 2017

09:00-09:30 **Late Registrations**
09:30-10:00 **Opening Ceremony under the patronage of the Tunisian Minister of Higher Education and Scientific Research: Pr. Slim Khalbous and the local committee: Pr. Manef Abderrabba, Pr. Ali Ayadi and Dr. Ines Sifaoui**
10:00-11:00 **Keynote Lecture 1 by Govinda Visvesvara, Human and animal infections caused by Free-Living amoebae- a retrospective**
(Chair: Ines Sifaoui & Jacob Lorenzo-Morales)

11:00-11:20 Coffee Break

11:20-12:50 **Oral Session 1**

Or-1: The Ameba and Its Ameba-Devouring Co-Infectant Were Recovered From A *Balamuthia* Encephalitic Survivor

Or-2: Acute meningoencephalitis due to a Free living Protozoa (amoeba-ciliate) N. G., N. Sp., and *Streptococcus pneumoniae*

Or-3: Sub-acute meningoencephalitis due to free-living amoeba [*Insertae Sedis]*

Or-4: Natural infestation by a Primary Amoebic Meningoencephalitis in cattle and sheep in the East of Algeria

Or-5: Primary amoebic meningoencephalitis in a dog in the Canary Islands

Or-6: Primary Amoebic Encephalitis Preventive Nose Plugs: Prophylaxis against *Naegleria fowleri* Infection

13:00-14:30 Lunch

15:00-16:00 **Oral Session 2** (Chairs: Thelma Dunnebacke & Yann Héchard)

Or-7: A Proposed Cascade Of Vascular Events Leading To Granulomatous Amoebic Encephalitis

Or-8: *Naegleria gruberi* selenocysteine pathway and cell growth modulation triggered by selenium intake

Or-9: An Antifungal Drug as the Candidate for Treatment of Primary Amebic Meningoencephalitis

Or-10: Successful Treatment of Primary Amoebic Meningoencephalitis using a novel therapeutic regimen including Miltefosine and Voriconazole

16:00-16:20 Coffee Break

16:20-18:00 **Oral Session 3** (Chairs: Maritza Omaña & Naveed A. Khan)

Or-11: Occurrence And Molecular Identification Of Free-Living Amoebae In Italian Geothermal Water Sorces

Or-12: *Balamuthia mandrillaris* isolated from commercial garden soil in store in Lima-Peru

Or-13: Presence of *Balamuthia mandrillaris* in hot springs from Mazandaran province, northern Iran

Or-14: Pathogenic Waterborne Free-Living Amoebae: an Update from Selected Southeast Asian Countries

Or-15: *Vermamoeba vermiformis* strains isolated from environmental sources from Canary Islands.

Or-16: Detection of Free-living amoebae in southern regions of Tunisia

THURSDAY 13 APRIL 2017

***Acanthamoeba* keratitis Awareness day**

09:30-10:30 **Keynote Lecture 2 by Dr. Julia Walochnik, Medical University of Vienna, Austria, *Acanthamoeba* as host and vehicle for bacteria and fungi**

(Chair: Fathi Moussa and Jacob Lorenzo-Morales)

10:30-11:15 **Oral Session 4**

Or-17: Treatment of *Acanthamoeba* keratitis

Or-18: Epidemiological features and risk factors in *Acanthamoeba* keratitis: about six cases

Or-19: Spectrum of microbial keratitis in the Gap Bon region of Tunisia

11:15-11:35 Coffee Break

11:35-12:35 **Oral Session 5** (Chairs: Manef Abderrabba & Ruqqaiyah Siddiqui)

Or-20: *Acanthamoeba* keratitis. Report of 3 cases diagnosed in southern Tunisia

Or-21: Investigation of the prevalence of *Acanthamoeba* keratitis in Turkey

Or-22: *Acanthamoeba-Fusarium* mixed keratitis in a Spanish contact lens user

Or-23: Failure of molecular detection of keratitis inducing *Acanthamoeba* strains in diagnostics

13:00-14:30 Lunch

14:30-16:00 **Oral Session 6** (Chairs: Julia Walochnik & Ruqqaiyah Siddiqui)

Or-24: Organ lysates of crocodile possess antimicrobial and antitumor activities

Or-25: *In vitro* activity of 1H-Phenalen-1-one derivatives against *Acanthamoeba castellanii* Neff and its mechanisms of cell death.

Or-26: Chlorine dioxide induces the amoebicidal effect on pathogenic *Naegleria fowleri, Acanthamoeba castellanii* and *A. polyphaga*

Or-27: Evaluation of the Anti-*Acanthamoeba* activity of two commercial eye drops commonly used to lower eye pressure

Or-28: Evaluation of Amoebicidal effects and cytotoxicity of multipurpose contact lens disinfecting solutions in Korea

Or-29: Status of the effectiveness of contact lens solutions against keratitis-causing pathogens

16:00-16:20 Coffee Break

16:20-17:20 **Oral Session 7** (Chairs: Atteneri López-Arencibia & Govinda S. Visvesvara)

Or-30: Human Innate Immunity and *Acanthamoeba*: effect of oral hormonal contraception

Or-31: Qualitative analysis of human monocyte and macrophage proteomes after stimulation with *Acanthamoeba* soluble products: a preliminary study

Or-32: Detection of serum antibodies in children and adolescents against *Balamuthia mandrillaris*, *Naegleria fowleri* and *Acanthamoeba* T4

Or-33: *Balamuthia mandrillaris* coexisting with other thermophilic amoebae in a natural source of thermal water

17:20-18:15 **Poster Session 1** (from 1 to 15) 3 minutes flash presentation a sort of advertising the poster and then time to network with the poster presenters (Chairs: Maritza Omaña Molina & Jacob Lorenzo Morales)

18:15 **Presentations from candidates to host FLAM2019**

FRIDAY 14 APRIL 2017

09:30-10:30 **Keynote Lecture 3 by Dr. Naveed Khan, Sunway University, Malaysia**
"**Brain-eating amoebae: Emerging problem with fatal consequences**
(Chairs: Ali Ayadi & Albrecht Kiderlen)

10:30-11:30 **Oral Session 8**

Or-34: Mitochondrial genome of *Acanthamoeba* and the relevance of genotype assignment

Or-35: Analysis of the nuclear small subunit ribosomal RNA gene of *Acanthamoeba* and identification of two novel genotypes in Thailand

Or-36: Evidence of a M1-Muscarinic GPCR homolog in unicellular eukaryotes: featuring *Acanthamoeba* spp. Bioinformatics 3D-modelling and Experimentations

Or-37: The Status of Molecular Analyses of *Acanthamoeba* Isolates Maintained by International Culture Collections

11:30-11:50 Coffee Break

11:50-13:10 **Oral Session 9** (Chairs: Patrick Scheid & Antonella Mattana)

Or-38: *Mycobacterium llatzerense*, a new amoebae resisting bacteria found in drinking water

Or-39*:* *Legionella pneumophila* prevents proliferation of *Acanthamoeba castellanii*

Or-40: Description of the early events of *Acanthamoeba culbertsoni* invasion in the model of granulomatous amoebic encephalitis (GAE) in diabetic mice

Or-41: First Report Of *Vermamoeba vermiformis* In Clinical Samples From Patients In Venezuela.

Or-42: *Acanthamoeba castellanii* is a potential host for *Streptococcus pyogenes* and *Streptococcus pneumonia*

13:10-14:30 Lunch

14:30-15:30 **Oral Session 10** (Chairs: Fernando Lares & María Reyes-Batlle)

Or-43: Amoebicidal activity of α-bisabolol against the trophozoite stage of *Acanthamoeba castellani* Neff

Or-44: Antiparasitic activity of seaweed extract from the Tunisian coast

Or-45: *In vitro* study of *Acanthamoeba culbertsoni* isolated from a clinical case with intraocular dissemination

Or-46: In vivo CNS infection model of Acanthamoeba genotype T4: the early stages of infection

Or-47: Evidence Targets of Aminodarone in *Acanthamoeba* spp.Bioinformatics and experimentations.

15:30-15:50 Coffee Break

15:50-17:00 **Poster Session 2** (from 16 to 31) 3 minutes flash presentation a sort of advertising the poster and then time to network with the poster presenters (Chairs: Maritza Omaña Molina & Jacob Lorenzo Morales)

*** Oral Communication: 15-20 minutes (max 15 minutes presentation and 3 minutes questions)

Poster Communication

P-1: Primary Amoebic Encephalitis Preventive Nose Plugs: Prophylaxis against *Naegleria fowleri* Infection

P-2: A Proposed Cascade Of Vascular Events Leading To Granulomatous Amoebic Encephalitis

P-3: Evidence of a M1-Muscarinic GPCR homolog in unicellular eukaryotes: featuring *Acanthamoeba* spp. Bioinformatics 3D-modelling and experimentations.

P-4: Evidence Targets of Aminodarone in *Acanthamoeba* spp. Bioinformatics and experimentations.

P-5: *Acanthamoeba castellanii* secreted proteins enhance IL-10 production through the protein kinase A-dependent pathway in human monocytes

P-6: Oral contraceptives modulate the anti-inflammatory activity of *Acanthamoeba*-stimulated human macrophage

P-7: Detection Of *Naegleria* Species In Geothermal Springs In Italy

P-8: Interactions of *Salmonella* Typhimurium mutants with amphizoic amoeba *Acanthamoeba castellanii*

P-9: *Acanthamoeba* (T4) increases paracellular permeability and transepitelial resistance by modifying tight juntions composition without change MDCK epitelial cells morphology

P-10: Contact lens-related infectious keratitis: review of 29 cases from Tunisia

P-11: *Acanthamoeba* spp. Detection And Molecular Characterization In Stray Cats From Madrid, Spain.

P-12: Comparison of Acanthamoeba PCR assays on human corneal samples for the diagnosis of Acanthamoeba keratitis
P-13: Detection of free-living amoebae isolates from regional hospitals in the Sierra Norte and Central Valleys of Oaxaca, Mexico
P-14: Epigenetic regulators for encystation of *Acanthamoeba castellanii*
P-15: Fatal granulomatous amoebic meningoencephalitis due to free living amoebae in two boys in two hospitals different in Lima- Peru
P-16: Free-living amoebae isolated from extreme environments
P-17: Free-living amoebae isolated from a lagoon Chinchaycocha in the highlands of the central Andes of Peru
P-18: The DNA databases for the genus *Acanthamoeba*; an update to 2017
P-19: Transcriptomic analysis and profiling of differential expression genes between *Naegleria fowleri* trophozoite and cyst
P-20: The identification of *Legionella* spp. and free-living amoebae (FLA) in water supply systems of hospitals by MALDI-TOF analysis and sequence-based typing (SBT)
P-21: Molecular isolation of free-living amoebae from Namhangang (South Han Liver) in South Korea
P-22: Isolation and biological characterization of *Acanthamoeba castellanii* from a case of keratitis in Mexico
P-23: Identification of *Acanthamoeba* genotypes in the cornea samples of wild birds
P-24: Identification of free-living amoebae isolated from tap water in Istanbul, Turkey
P-25: Prevalence of Free living amoebae in the domestic water reservoirs in Sfax, Tunisia
P-26: Molecular characterization of free amoebae responsible for Keratitis diagnosed at the La Rabta hospital in Tunis
P-27: Anti-*Acantamoeba* activity of citrus peels essential oil and the effect of viroids infection
P-28: Anti-*Acantamoeba* activity of *Melalleca styphelioides* extracts
P-29: Activity of *Ammoides pusilla* essential oil and extracts against *Acanthamoeba castellanii* neff
P-30: Studies on essential oil composition and anti-*Acanthamoeba* activity of *Teucrium ramosissimum*
P-31: Anti-*Acantamoeba* activity of *Thymus Capitatus* essential oil and extracts

*** Poster session will include 3 minutes flash presentation and time to network with the poster presenters.

KEYNOTES

Keynote Lecture 1: Human and animal infections caused by Free-Living amoebae- a retrospective

Govinda S. Visvesvara

(Retired) from the Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA

Small free-living amoebae (FLA), belonging to the genera *Acanthamoeba*, *Balamuthia* and *Naegleria* cause fatal central nervous system (CNS) infection in humans and other animals, most often leading to death. Several species of *Acanthamoeba* and *Balamuthia mandrillaris* cause an insidious and chronic granulomatous amebic encephalitis (GAE), and skin infections. Both *Acanthamoeba* spp. and *B. mandrillaris* have been frequently identified in immune-compromised persons, either because of HIV/AIDS or organ transplantation. Additionally, *Acanthamoeba* spp. also cause vision-threatening infection of the human cornea, *Acanthamoeba* keratitis (AK). *N. fowleri* is known to cause an acute, fulminating infection, primary amebic meningoencephalitis (PAM). CNS infections caused by these three amoebae lead generally to death although a few patients have survived. Additionally, *Sappinia* sp., another FLA has also been identified as the agent of amebic encephalitis in a patient who survived the infection. Although vahlkampfiid amoebae have been implicated in causing amebic keratitis in humans, recently however a *Paravahlkampfia* sp. was isolated from corneal specimens and a new species *P. francinae* was isolated from the cerebropspinal fluid of a patient with typical symptoms of PAM but recovered from the infection.
Acanthamoeba spp. and *B. mandrillaris* also cause infections of the CNS of animals including gorillas, monkeys, dogs, ovines, bovines, horses, and kangaroos and birds. *Acanthamoeba* spp. have also been isolated from the tissues of reptiles, amphibians, fishes and even invertebrates. Based on sequencing of the SSUrRNA gene, of several *Acanthamoeba* isolates from fish, reptiles, birds and those associated with human *Acanthamoeba* keratitis infections, belong to T4 genotype, suggesting that features that enable these amoebae to infect animals, may also help them to infect humans. *Vahlkampfia* sp. along with *Acanthamoeba* and *Hartmanella* has also been isolated from domestic mammals and birds. Diseases caused by these amoebae have received little attention because of lack of suspicion and limited diagnostic expertise coupled with limited resources.

Keynote Lecture 2: *Acanthamoeba* as host and vehicle for bacteria and fungi

Julia Walochnik

Molecular Parasitology, Institute for Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Austria

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Objectives : Owing to their ubiquity, their resilience and their extremely robust cysts, the representatives of the genus *Acanthamoeba* are of particular importance as vehicles for other microorganisms, harbouring them inside their cells and protecting them from adverse environmental conditions. Besides legionellae, numerous other potential pathogens have been demonstrated to survive or even multiply within *Acanthamoeba* spp., including among others *Burkholderia* spp., *Chlamydia* spp., *Listeria monocytogenes*, *Mycobacterium* spp. and *Vibrio cholerae*. The aim of this overview is to further complete the picture of *Acanthamoeba* as a host organism.

Methods: Over the past years, in several projects with numerous collaborators, we screened water and soil samples from various habitats for *Acanthamoeba* and other free-living amoebae and further screened the isolated amoebae for intracellular bacteria and fungi, both by culture and molecular techniques.

Results: We have demonstrated that *Acanthamoeba* is the predominant amoebozoan genus in Austrian engineered waters, its presence and viability not being affected by regular disinfection and correlating with the occurrence of pseudomonads and legionellae. Further, we detected an unusually high abundance and diversity of *Acanthamoeba* spp. in soil from African regions endemic for melioidosis, one *Acanthamoeba* isolate being permanently infected with *Burkholderia pseudomallei.* Moreover, we have confirmed that *Acanthamoeba* is not only a suitable host but also a suitable prey for several fungi.

Conclusions: In recent years, several studies, including our own ones, have shown that a high percentage of environmental and also of clinical isolates of *Acanthamoeba* naturally harbour diverse intracellular microorganisms, occasionally also of more than one species at the same time. Finally, we provided the first confirmation for a natural long-term "symbiosis" between *Acanthamoeba* and *Burkholderia pseudomallei,* the causative agent of melioidosis.

Keynote Lecture 3: Brain-eating amoebae: Emerging problem with fatal consequences

Ruqaiyyah Siddiqui, Timothy Yu Yee Ong, Naveed Ahmed Khan*

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Pathogenic free-living amoebae (*Acanthamoeba*, *Balamuthia mandrillaris*, *Naegleria fowleri*) can cause central nervous system infections that almost always result in death, despite advances in available diagnostic tools, antimicrobials chemotherapy and supportive care. Moreover, the pathogenesis and pathophysiology of amoebic infections remains incompletely understood and it is not clear how amoebae invade the brain to attack the central nervous system, leading to neuropathogenesis. Here, we summarize amoebae infection of the central nervous system emphasizing the shortcomings of useable therapies and discuss needs for future research. A better understanding of pathogenesis and pathophysiology, diagnosis, potential drugs, and mechanisms of action will facilitate the development of more effective chemotherapies.

ORAL PRESENTATION

Or-1: The Ameba and Its Ameba-Devouring Co-Infectant Were Recovered From A *Balamuthia* Encephalitic Survivor

Thelma Dunnebacke

Viral and Rickettsial Disease Laboratory, California Department of Public Health, Richmond California <dunnebacke@gmail.com>

This investigation began as an examination of biopsy tissues from a patient following surgery for a suspected brain tumor. Based on laboratory test, confirmed at CDC, the diagnosed was found to be *Balamuthia mandrillaris* encephalitis. Following the recommended treatment with anti-fungal drugs, the patient recovered and continues to lead a full and active life.

Recovery of the ameba from the biopsy tissues was complicated by an early outgrowth on mammalian cell sheets of a small unexpected (unex) cell that initially cloaked the activity and recognition of the amebae. Subsequently, recovery and separation of the unex and the amebae led to findings of interactions between these two very different cells when they were placed together in a culture dish. Active amebae were seen to approach the smaller, unmoving unex, gather it within its pseudopodia then release a squashed crumpled unex shell. The ameba may have fed on the unex content. More commonly, their interactions involved the active unex moving to and attaching onto the body of an ameba, where, they were joined by other unex, and stayed for a number of hours as the ameba was digested. The unex did not attack Balamuthia cysts, nor did they attack an destroy Acanthamoeba.

Both the amebae and the unex cells have been microscopically and immunologically identified within the patients' sectioned brain material. They were located in separate, as well as intermingled in areas of the brain tissue. Conjunctive unex/ameba complexes, observed in live cultures as the amebae were digested by the unex, were also present in areas of the parenchyma and, in particular, within the lumen of blood vessels.

Our observations are suggestive that the unex and the amebae were co-invaders, perhaps entering the brain via the vascular system. Although the sequence analysis of the unex cells is not yet complete, the unex morphology and growth conditions are compatible with that of a dimorphic carnivorous fungus.

Or-2: Acute meningoencephalitis due to a Free living Protozoa (amoeba-ciliate) N. G., N. Sp., and *Streptococcus pneumoniae*

Alfonso M. Cabello-Vílchez*, Enrique Silva-Tica, Daniel Guillen-Pinto, Eduardo Gotuzzo & Dalila Y. Martínez. *Alfonso M. Cabello-Vílchez
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Acute meningoencephalitis due to free-living protozoa is a very uncommon disease, almost fatal, which does not have a specific therapy. We describe an 11 year-old boy with 5 days history of fever, chills and asthenia. A couple of days later, he developed progressive headache and vomiting. Finally, he had hyporexia, hypersomnia, neck pain and irritability. He was admitted with the diagnosis of acute meningoencephalitis and empiric therapy was installed with Ceftriaxone and Vancomycin, after performing blood cultures. A brain computed tomography (CT) scan showed ventriculomegaly and diffuse edema, MRI showed hypointense lesions in basal ganglia and hyperintensity in cerebellum hemispheres that enhanced with contrast infusion. Six hours after admission, he became incoherent with involuntary movements. He was admitted to intensive care unit (ICU), because he shocked and went into coma. Lumbar puncture showed a cerebrospinal fluid (CSF) with high pressure, pleocytosis, hyperproteinorrachia and hypoglycorrhachia; Gram, acid-fast stain and Indian ink were negative; coaglutinations and polymerase chain reaction (PCR) for *Streptococcus pneumoniae* were positive. A wet mount of CSF showed mobile trophozoites (5 – 6 by field) of 18-25µm. Patient had traveled to Iquitos, Peru (Amazon jungle) 6 weeks before, where he swam and dive in fresh water pounds and pools. Anti-amebic medication was added to the antibiotics therapy, including miltefosine, albendazole, fluconazole, azithromycin and Amphotericin B deoxycholate. The patient improved in the following weeks (clinically and parasitic load); although, had developed relapses with increased of parasitic load. Free-living protozoa grew in cell culture, but short time. However PCR for known free-living amoebas were negative. This protozoan has a particular biological cycle that includes an amoeboid and a ciliated-membranous stage, a cyst stage has not been observed. It didn´t grow in agar nonnutritive or axenic culture with fetal bovine serum FBS 10%. This would be the first case of a meningoencephalitis caused by a new pathogenic protozoon for humans in partnership with a bacterium such as *S. pneumoniae*.

Or-3 : Sub-acute meningoencephalitis due to free-living amoeba [*Insertae Sedis]*

Dalila Y. Martínez*, Daniel Guillen-Pinto, Enrique Silva-Tica, Elmer Zapata-Yarleque, Iván Espinoza, Alejandro Álvarez, Eduardo Chaparro, Eduardo Gotuzzo, & Alfonso M. Cabello-Vílchez

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Free-living amoeba sub-acute meningoencephalitis is a rare and mostly fatal disease. Recognized agents in this group include: *Naegleria fowlery, Acanthamoeba sp., Balamuthia mandrillaris* and *Sappinia dipploidea*. Here we report two children with the history of headache, fever, sensorial hallucinations (olfactory and visual) and seizures. Case 1 corresponds to a 12 years-old girl with four weeks of symptoms at the admittance and meningeal signs on the physical exam. Complete blood count showed high neutrophilia, magnetic resonance images (MRI) showed diffuse edema. A lumbar puncture (LP) showed a high opening pressure, with pleocytosis, hypoglycorrhachia, and hyperproteinorrachia on her cerebrospinal fluid (CSF). Gram, acid-fast stain and Indian ink were negative. Empiric therapy with Acyclovir, Ceftriaxone and Vancomycin was installed. Two weeks later, patient didn´t improve, several test for infections and autoimmune diseases were negative. A new LP showed the presence of 20 trophozoites (~ 25- 100 µm) in 1 field of 100x in a wet mount test. She was a semiprofessional swimmer. Case 2 corresponds to an 8 years-old girl with three weeks of symptoms, slight decrease on the strength of her left body side and meningeal signs. An MRI showed the presence of hyperintensity in her right frontal-parietal cortex. The LP showed pleocytosis, hypoglycorrhachia, and hyperproteinorrachia. Gram, acid-fast stain and Indian ink were negative, as well as other test for infectious diseases. In the wet mount of CSF, two trophozoites (~ 9 -12 µm) in 1 field of 100x were observed. Later on, she started to lose her vision. In both cases, an empiric therapy including amphotericin B deoxycholate, miltefosine, albendazole, fluconazole and azithromycin or sulfamethoxazole/trimethoprim was installed with clinical and MRI improvement after one month of therapy. They continue in maintenance therapy until now. None of the identified amoebas grew on non-nutrient agar plates coated with *E.coli.* The morphology of these trophozoites were different and very particulars, they didn´t form cysts. Polymerase chain reaction 18S was negative in both cases. We describe two cases of subacute meningoencephalitis caused by none of the pathogenic free-living amoeba *[Insertae Sedis]* that and improved with a combination of empiric anti-amoebic therapy.

Or-4: Natural infestation by a Primary Amoebic Meningoencephalitis in cattle and sheep in the East of Algeria

A.Ayachi[1] ¨ M.S. Benterki[2]., O.Bennoune[3]., N.Heleili[1]., M.Pelandakis[4]

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A natural infection by *Naegleria fowleri* occurred in ruminants in hot summer , one cow and one ewe have been contaminated with drinking warm water. This disease was clinically diagnosed by discarding all another causes of meningoencephalitis in ruminants

Objectives: A deep study of this reported case was carried out in order to diagnose the etiology of this meningo encephalitis

Methods: The acute and fatal disease was described clinically in both animals and paraclinically by haematological and biochemical exams. In addition May-Grünwald-Giemsa (MGG), and Methylene blue staining of fresh and refrigerated Cerebro Spinal Fluid (CSF) was performed. The oocyte was isolated from CSF by culture of trophozoites on non-nutritive Agar and flagellation Test (FT) was done. An inoculation of a bolus of trophozoites to hamsters was executed. Histologic smears of brain stained with Hematoxylin and Eosine (H&E) was also completed. DNA PCR of this parasite was made to identify its specie.

Results: All clinical study and lab exams lead us to suspect a parasitic disease. The CSF, after staining with MGG, showed the presence of amoebae cells. The FT showed a motile trophozoite belonging to *Naegleria* genus. The histological sections revealed numerous amoebae in all parts of the brain. The experimental infestation of hamsters by a bolus of trophozoites finished by their death in 10 day. The Real time PCR carried out on the DNA parasite, ended with the appearance of specific bands to *Naegleria sp*. The sequencing that allows determining the specie of the parasite leads us to *Naegleria fowleri*

Conclusion: Particular attention should be focused on this type of infection in aquatic environments when the temperature is high and preventive measures must be taken to avoid the proliferation of *N. fowleri*

Or-5: Primary amoebic meningoencephalitis in a dog in the Canary Islands

María Valladares [1,2], María Reyes-Batlle[2], Johanna González Mendez[3], Atteneri López-Arencibia[2], Ines Sifaoui[2], Enrique Martínez-Carretero[2], José E. Piñero[2], Basilio Valladares[2], Jacob Lorenzo-Morales[2]

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The first case of canine Primary Amoebic Encephalitis in the Canary Islands is described in this work. A young beagle was admitted to the veterinary clinic showing cervical pain and signs of lethagia and vomitting. Direct examination of samples submitted to the laboratory of free living amoebae in Tenerife, highlighted the presence of trophozoites compatible to Naegleria genus.

After specific PCR/sequencing, the amoeba was diagnosed as a member of Naegleria fowleri species. At this stage, dog was treated with a combination of drugs including a cocktail of rimfapicin, fluconazole and miltefosine. Even though the dog started to recover at this stage, a small ulcer in the nose was observed as well as cervical pain. After 20 days of treatment with miltefosine, PCR and culture was repeated and was negative. Moreover, the dog was showing almost full recovery and currently no remission has been observed. Therefore, this is the first time that a sucessfull treatment is applied in a canine case of PAM.

Acknowledgments: This work was supported by the RICET grants (project RD12/0018/0012 of the programme of Redes Temáticas de Investigación Cooperativa, FIS), Spanish Ministry of Health, Madrid, Spain; and Project BIO24 "Principios activos inductores de apoptosis en la quimioterapia de tripanosomosis y leishmaniosis" (project 2016_25) from Obra Social La Caixa-Fundación CajaCanarias. ALA.and IS were funded by the Agustín de Betancourt Programme.

Or-6: Primary Amoebic Encephalitis Preventive Nose Plugs: Prophylaxis against *Naegleria fowleri* Infection

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Objective: Naegleria fowleri is a free-living amoeba; it is a protist pathogen that is known to cause a fatal encephalitis in humans known as "Primary Amoebic. Mostly, PAM is reported in recent swimmers and people who perform ablution and nasal cleansing. Much has been done for vaccination and treatment without any success in past 60 years. One of the most alarming aspects of PAM is the mortality rate that has remained greater than 99% (1) in past 60 years Despite our advances in anti-amoebic chemotherapy, the treatment modalities are mostly empirical, which often results in deaths (2). Some drug delivery and diagnostic devices (3) have been proposed recently, but they are yet to be implemented. We propose a prophylaxis for this disease by introducing a device "Naeglerіopel". This device is non-invasive and requires insertion into the nostrils at times of swimming or water sports related activities.

Methods: For prophylaxis of PAM, we propose a device called **"Naeglerіopel"** that would block the pathogen at its portal of entry to the nose at the nostrils. This detachable device can be designed to be comfortable, cosmetically acceptable and easy to wear. Made up of synthetic plastic or silicone, this device would be adapted to the contours of the interior of the nose. As a nasal plug, this device with a convex round contoured tip and a hollow cavity could be easily inserted into the nostril bilaterally. The free ends of this connector bridge would be made to reach the centre of the concavity of a pair of nasal clogs. Each nasal clog would be made-up of expandable semi-firm, but of a soft silicone. These mushroom shaped nasal clogs connected at their centre of concavity to the central bridge would make the device insertable into the nostrils bilaterally. The connector ends could be glued or screwed to the clogs to prevent detachments. This device could be manufactured in three different sizes to suit different sizes of nasal apertures and age groups, which would enable its use in both, children and adults. The outer surfaces of the nasal inserts would be kept smooth and glistening to avoid irritation and subsequent sneezing or discomfort.

Results: This method is robustly expected to prevent the chance entry and access of Naegleria fowleri to the upper parts of the nasal cavity and lower down the scare of underwater swimming by preventing the entrance of contaminated water into the nose. It would help prevent the coughing and sneezing secondary to the water entry into the nose in individuals who are amateur swimmers and occasional swimmers. In areas of high incidences its free availability is expected to remarkably lower down the cases occurring annually.

Conclusion: This proposal could be tested in more human volunteers and could be evaluated for its compliance. Given the fact that Naegleria Fowleri has a mortality rate close to 99%, this device could offer a real protection of from the PAM and reduce the anxiety associated in the midst of swimming with head under the water.

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Or-7: A Proposed Cascade of Vascular Events Leading To Granulomatous Amoebic Encephalitis

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Objective: Granulomatous amoebic encephalitis due to *Acanthamoeba* is a chronic disease that almost always results in death (1). Hematogenous spread is a pre-requisite followed by amoebae invasion of the blood-brain barrier to enter the central nervous system (2).
Given the systemic nature of this infection, a significant latent period of several months before the appearance of clinical manifestations is puzzling (3). Based on reported cases, here we propose pathogenetic mechanisms that explain the above described latency of the disease.
Methods: Review of literature with details of the pathogenesis was explored and a model of infection was constructed. The sequence of event of *Acanthamoeba* in cerebral circulation was constructed.
Results: It was inferred that *Acanthamoeba* uses adhesion molecules and integrins to attach and invade the blood brain barrier and infect the brain. Also, there appears to be chemical chemotaxis the directs this protist towards the brain.
Conclusion: Knowledge of the pathogenetic steps involved in GAE could help manufacture drugs that could resist the CNS invasion after infection of soft tissue and skin by *Acanthamoeba* spp.

Keywords: GAE, Acanthamoeba, Blood Brain Barrier

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Or-8: *Naegleria gruberi* selenocysteine pathway and cell growth modulation triggered by selenium intake

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Naegleria spp. besides the human pathogenic *N. fowleri*, includes free living amoebas that in order to overcome adverse environmental conditions can change to flagellate and cystic forms and are widely distributed around the world. Here we are interested in *Naegleria gruberi*, a non-pathogenic specie as a model to elucidate the biosynthesis pathway of selenocysteine (Sec) of these amoeba-flagellates. Sec is co-translationally incorporated in the nascent peptide at a UGA codon dependent on several specific factors not fully investigated in eukaryotes. The discovery of two atypical gene fusions in the essential protein selenophosphate synthetase (SPS2), that catalyzes selenide and adenosine triphosphate (ATP) conversion into selenophosphate required for the Sec synthesis, with metyltransferase (*Ng*SPS2.MT) and a NifS like protein (*Ng*SPS2.NifS) is unclear and it is being investigated. In order to interrogate the function of these gene fusions we are performing sodium selenite supplementation analysis in ATCC 1034 medium.

Our results show a dual behavior ranging from cell growth improvement (5 µM) to cell growth inhibition (25 µM) depending on the selenium concentration. The growth curves also reveal that the cells become less sensitive to stress agent – hydrogen peroxide - associated with selenium intake, which seems to correlate with an augmented Sec pathway activity. To assess the *Ng*SPS2.MT and *Ng*SPS2.NifS contributions, both gene transcripts have been quantified by Real-Time PCR alongside an analysis of several *N. gruberi´*s potential reference genes and Sec pathway genes. We have determined that *Ng*SPS2.MT is post-translationally cleaved generating separate MT and SPS2 domains. Currently we are investigating if the same happens with *Ng*SPS2.Nifs fusion. Differential intracellular localization of both *Ng*SPS2.MT and *Ng*SPS2.Nifs is also being investigated. Our results should contribute to the understanding

Or-9: An Antifungal Drug as the Candidate for Treatment of Primary Amebic Meningoencephalitis

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Optimum treatment for primary amebic meningoencephalitis (PAM), caused by a free-living ameba *Naegleria fowleri*, has not been defined. Only about a dozen patients have been treated successfully worldwide with amphotericin B or miltefosine, either alone or in combination with other drugs. Therefore, development of efficient drugs is a critical unmet need to prevent future deaths of children and young adults.

Objective: *De novo* biosynthesis of ergosterol in *N. fowleri* occurs via cycloartenol, a sterol precursor of fungi and plants. Sterol biosynthesis is a validated target in fungi because sterols are ubiquitous components of the plasma membrane. Since successful infection by *N. fowleri* is dependent on adherence to host cells and *N. fowleri* membrane plays an important role in this process, we hypothesized that disruption of sterol biosynthesis by small molecule inhibitors may provide an effective treatment option for PAM.

Methods: Sterol 14-α-demethylase (CYP51) plays an essential role in sterol biosynthesis and azoles are known CYP51 inhibitors. We tested the activity of different azoles on *N. fowleri* and chemically validated sterol biosynthesis as a drug target by assessing the effect of an azole on the composition of endogenous sterols of *N. fowleri*. Finally, we characterized drug-target interaction between *N. fowleri* CYP51 and an azole drug by UV-vis spectroscopy and X-ray crystallography.

Results: Our cell-based assay identified amebicidal activities of four azole drugs, posaconazole, ketoconazole, clotrimazole and miconazole, with EC50 ranging from 0.2 μM for posaconazole and ketoconazole to 2.6 μM for miconazole. Both posaconazole and ketoconazole have similar EC50 to amphotericin B and all compounds showed much higher potency than miltefosine, a currently recommended drug. We focused on posaconazole for follow up studies because of less toxicity and availability of both oral and intravenous formulations. Sterol mixtures extracted from 24-h posocanozole-treated *N. fowleri* trophozoites identified dramatic increase of 31-norlanosterol and concomitant decline in ergosterol compared to the untreated trophozoites. We determined the crystal structure of *N. fowleri* CYP51 and posaconazole complex with 1.7 Å resolution.

Conclusions and significance: Our work chemically validated the sterol biosynthesis pathway in *N. fowleri* as an attractive drug target and identified an FDA-approved antifungal drug posaconazole as a new candidate for the treatment of PAM.

Or-10: Successful Treatment of Primary Amoebic Meningoencephalitis using a novel therapeutic regimen including Miltefosine and Voriconazole

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Primary amoebic meningoencephalitis (PAM) is an uncommon disease and most-likely fatal disease, caused by *Naegleria fowleri* for which there is no optimal therapy. We describe an 8-year-old boy with a 24 hours history of progressive headache, nauseas, and fever (38.5°C). The patient had been taken swimming lessons three times by week during the last month in a public pool. The initial evaluation was normal while waiting the laboratory test the headache impaired and turned sleepiness. A nasal swab showed suggestive forms of trophozoites. A brain computed tomography and magnetic resonance image (MRI) with and without contrast, yielded diffuse cerebral edema without focal enhanced lesions. Lumbar puncture was performed and the cerebrospinal fluid (CSF) showed pleocytosis (80% neutrophils), hyperproteinorrachia, and normal glucose level. Gram stain, acid-fast, and Indian ink didn´t show any organism. A wet mount of the CSF revealed 1-3 trophozoite (9-12µm) in 1 field of 100x, suggestive of *Naegleria sp*. Patient´s neurologic status deteriorated quickly, he showed clear meningeal signs (Kernig´s and Brudzinsky´s sign), bilateral Babinsky, photophobia, and alteration of brain superior functions.

Empirical therapy was started with amphotericin B deoxycholate (cumulative dose, 720 mg), voriconazole (325 mg per day), miltefosine (75 mg per day), and rifampin (360 mg per day). Intracranial hypertension was managed initially with mannitol (54 gr per dose) and dexamethasone (5.4 mg per dose) in the first 36 hours. Later on mannitol was switched by hypertonic saline. Five-days later, symptoms have mild improvement (headache and concentration) and fever was solved. A new MRI showed resolution of brain edema, and dexamethasone and hypertonic saline were discontinued; voriconazole was switched to PO, and azithromycin was added (360 mg per day). A new CSF study after 20 days of therapy was reported as normal and patient have regression of all neurological symptoms. He was discharged home with the prescribed antibiotics until complete 28 days of therapy and without any disability or sequela. He has no recurrence or relapse until now (One year of follow-up). We describe the first young survivor with PAM who had complete recovered after receiving a regiment including miltefosine and voriconazole among others drugs.

Or-11: Occurrence And Molecular Identification Of Free-Living *Amoebae* In Italian Geothermal Water Sorces

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Objectives: In the present study a survey was conducted in order to detect and to characterize at molecular level free-living amoebae (FLA) from different geothermal water sources in Italy, to investigate their ecology and epidemiology.

Methods: Water samples were taken on a quarterly basis for one year (April 2015 -July 2016), in two geothermal hot springs (site A and B) located in Latium Region. Eight sampling points were chosen for the whole area. Two-liter of water were collected for each point and processed within 48h. Water temperature and pH were recorded. Each sample was centrifuged and cultured onto Non Nutrient Agar-media containing Escherichia coli. All agar plates were incubated at different temperatures and observed daily for amoebic growth. Molecular characterization was obtained through DNA extraction and amplification using three sets of primers. To determine species/genotype a phylogenetic analysis using MEGA 6 was executed.

Results: A total of 38 water samples (site A=25; site B=13) were collected and analyzed. Twenty-nine out of 38 (76.31%) samples were positive for growth of FLA, twenty-three (92%) from the site A, six (46.15%) from the site B. Phylogenetic analysis of positive isolates allowed to identify, among Amebozoa *Vermamoeba vermiformis*, *Echinamoeba* spp., *Platyamoeba* sp., *Stenamoeba* sp. and *Acanthamoeba* (genotype T4, T15); among Excavata: Fumarolamoeba ceburocoi, *Vahlkampfia* spp., and *Naegleria* spp..

Conclusions and Significance of the work: The present ongoing study is the first molecular based investigation providing an overview about community composition and seasonal distribution of FLA in Italian geothermal waters. Interestingly, among the species/genotypes identified, genotypes T4 and T15 of Acanthamoeba are responsible for human keratitis as well as *V. vermiformis* and *Vahlkampfia* spp. more recently, while some Naegleria species can cause disease in humans and animals. The presence of potentially pathogenic amoebae in habitats related to human activities supports the relevance of FLA as potential public health concern.

Or-12: *Balamuthia mandrillaris* isolated from commercial garden soil in store in Lima-Peru

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Balamuthia mandrillaris was identified 31 years ago as a pathogenic free-living protozoon that can cause fatal Granulomatous Amebic Encephalitis (GAE) in humans and animals. Perú has reported 55 cases, the highest number in Latin America. *B. mandrillaris* is difficult to isolate and to culture. Only 9 (3.44%) of 261 reported environmental isolation attempts have been successful. Environmental factors may be responsible for the high proportion of GAE in Latin America.

In this preliminary study, we have found that the garden soil being sold to the public in Lima, the capital of Perú, contains *B. mandrillaris*. The garden soil was tested for free-living amoebae because it could have been the source for the infection of a 12-year-old girl who developed subacute meningitis due to other pathogenic amoeba and survived after prompt diagnosis and treatment (D. Martínez.et al., 2017 report). The soil was from a single source, and one of 6 samples from it yielded *B. mandrillaris*, while other free living amoebas were found in the rest. Characteristic *B. mandrillaris* trophozoites took about a month and a half to appear in agar plates originally seeded with *Escherichia coli*. Lares-Jiménez culture medium is being used to attempt axenization of this new *B. mandrillaris* strain. There is no legislation or sanitary code in Perú regulating the trade of garden soil to control the potentially pathogenic microorganisms it could carry. We propose a larger study to confirm and extend these recent findings.

Or-13: Presence of *Balamuthia mandrillaris* in hot springs from Mazandaran province, northern Iran

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Objectives: *Balamuthia mandrillaris* is an opportunistic free-living amoeba that has been reported to cause cutaneous lesions and *Balamuthia* amoebic encephalitis. The biology and environmental distribution of B. mandrillaris is still poorly understood and isolation of this pathogen from the environment is a rare event. Previous studies have reported that the presence of *B. mandrillaris* in the environment in Iran may be common. However, no clinical cases have been reported so far in this country. In the present study, a survey was conducted in order to evaluate the presence of B. mandrillaris in hot-spring samples of northern Iran.

Methods: A total of 66 water samples were analysed using morphological and molecular tools. Positive samples by microscopy were confirmed by performing PCR amplification of the 16S rRNA gene of B. mandrillaris. Sequencing of the positive amplicons was also performed to confirm morphological data.

Results: Two of the 66 collected water samples were positive for *B. mandrillaris* after morphological and molecular identification. Interestingly, both positive hot springs had low pH values and temperatures ranging from 32 °C to 42 °C. Many locals and tourists use both hot springs due to their medicinal properties and thus contact with water bodies containing the organism increases the likelihood of infection.

Conclusions: To the best of our knowledge, this is the first report on the isolation of B. mandrillaris from hotspring sources related to human activity. Therefore, *B. mandrillaris* should be considered as a possible causative agent if cases of encephalitis are suspected following immersion in hot springs in addition to *Acanthamoeba* and *Naegleria*.

Or-14: Pathogenic Waterborne Free-Living *Amoebae*: an Update from Selected Southeast Asian Countries

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Abstract

Data on the distribution of free-living amoeba are still lacking globally, especially in the Southeast Asian region. The aquatic environment reveals a high occurrence of free-living amoeba (FLA) due to its suitable condition which subsequently causes infection to human via direct contact. We investigated the presence of pathogenic FLA in treated and untreated water samples from Laos (n=31), Myanmar (42), and Singapore (21). The FLA was cultured onto non-nutrient agar seeded with live suspension of *Escherichia coli* and incubated at room temperature. Morphological identification was conducted for both trophozoites and cysts via microscopic stains (Giemsa and immunofluorescence). The presence of *Naegleria*-like structures was the most frequently encountered in both treated and untreated water samples, followed by *Acanthamoeba*-like and *Vermamoeba*-like features. To identify the pathogenic isolates, species-specific primer sets were applied for molecular identification of *Acanthamoeba*, *Naegleria*, and *Vermamoeba*. The pathogenic species of *Acanthamoeba lenticulata* and *A. triangularis* were detected from untreated water samples, while *Vermamoeba vermiformis* was found in both treated and untreated water samples. Our results suggested that poor water quality as well as inadequate maintenance and treatment might be the cause of this alarming problem since chlorine disinfection is ineffective in eradicating these amoebas in treated water samples. Regular monitoring and examination of water quality are necessary to control the growth, hence, prevent widespread FLA infections among the public.

Or-15: *Vermamoeba vermiformis* strains isolated from environmental sources from Canary Islands.

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Free-living amoebae are ubiquitous protozoa commonly found in water and soil environments. Amoebae belonging to various genera, including Acanthamoeba, Balamuthia, Naegleria and Vermamoeba, can cause pathologies such as keratitis or meningoencephalitis. Some of them are also environmental carriers of other pathogenic agents such as bacteria and virus. The historical and best-known of those amoeba associated pathogen is Legionella pneumophila. Vermamoeba vermiformis has been reported from many locations worldwide and has been described to be a thermotolerant amoeba. V. vermiformis strains have been isolated from many habitats but the important to be highlighted is geothermal springs in countries such as Iran or Mexico among others.

Objectives: In this study, 60 samples from different environmental sources (snow, water and soil) of Canary Islands were collected and checked for the presence of FLA.

Methods: Samples collected were cultured directly on 2% non-nutrient agar (NNA) plates at 22°C and 37°C and were monitored daily for the presence of free-living amoebae with a layer of Escherichia coli suspension that had been heat-inactivated (2h at 60°C). Plates those were positive for amoebic growth were subcultured until was reached a clean plate.

Results: Morphological characterization of amoebic strains using the currently available identification key from Page yielded the identification of ten (16.7%) V. vermiformis strains from the 60 soil samples included in this study. All the isolates were able to grow at 22°C and 37°C.

Conclusions and significance of the work: All the isolated samples showed thermotolerance, which is an important pathogenicity factor. The presence of this species in water bodies in the islands should raise awareness to health authorities, due to the ability of this parasite to carry pathogenic microorganisms. Moreover and to the best of our knowledge, this is the first report of Vermamoeba vermiformis species in the Canary Islands.

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Or-16: Detection of Free-living amoebae in southern regions of Tunisia

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Free-living amoebae (FLA) are ubiquitous and opportunistic protozoa widely distributed in telluric and aquatic environments. They can cause opportunistic and non-opportunistic infections in humans and animals. In addition, these amoebas serve as hosts for a large number of pathogenic bacteria, yeasts and viruses.Our work was the first studies that are interested in axis of free amoebae identification and especially pathogenic genus *Acanthamoeba* spp and *Naegleria* spp in Tunisia following the discovery of the first case of keratitis in our laboratory. For this, we performed water sampling at the hospital (hemodialysis unit (46), dental unit (196), hospital circuit (84)) and environmental level (Oasis (211), reserves waters (486), thermal water sampling from the regions of Gabes, Medenine (53) and Sfax (63)).*Acanthamoeba* spp and *Naegleria* genus identification was carried out by PCR, PCR-RFLP and real time PCR. In the hemodialysis unit, FLA was detected in 97.8% of samples with predominance of acanthopodial morphotype (28.3%). *Acanthamoeba* spp was morphologically identified in 13 samples while the PCR amplification by JDP primers was positive for 15 samples. In dental units, FLA was detected in 69% of samples. The predominant morphotype was the monopodial (39.2%). 21 *Acanthamoeba* strains were isolated in these units (13.3%). the PCR amplification by P3-P4 primers was positive in 100% of cases. In hospital tap water, FLA was detected in 53.5% of samples with predominance of acanthopodial morphotype (88, 8%). The frequency of *Acanthamoeba* genus was 47, 6%. For molecular study, using Acan-F / Acan-R primers, *Acanthamoeba* was detected in 54 samples (64.2%). All *Acanthamoeba* keratitis (AK) were associated to T4 genotype. Three different DF3 sequence types were related to AK infections T4/10, T4/15, and T4/16.In environment water, *Acanthamoeba* was detected in 82% of oasis samples. Sequencing of the amplification products with primers P892C / P892 has allowed us to detect the predominance of T4 genotype (51%) with the presence of other genotypes.

In southern Tunisia, *Acanthamoeba* was present in 83% of samples and *Naegleria* in 57%. We also detected a combination of these two genus in 23 samples. In the Sfax region, the genus *Naegleria* was detected in 43% of the thermal water samples.The widespread occurrence of FLA in hospital water in the current study indicates that disinfection procedures and hygiene measures are insufficient to remove or destroy these protozoa. Therefore, monitoring the presence of FLA in hospital units and environment, as well as evaluating the pathogenicity of the isolates, can be an approach to alert the health professionals to improve the disinfection methods and minimize the risks from pathogenic FLA.

Or-17: Treatment of *Acanthamoeba* keratitis

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Acanthamoeba keratitis is a severe sight-threatening infection of the cornea. Early diagnosis and aggressive medical therapy is essential to improve its outcome. Topical drugs effective against trophozoites and cysts of Acanthamoeba are biguanides (PolyHexaMethyleneBiguanide, chlorhexidine) and aromatic diamidines (propamidine, hexamidine). These drugs are usually used in combination. No clear consensus exists about use of steroids. Therapeutic penetrating keratoplasty is dedicated for severe cases. We will review during our presentation the different features and issues of medical and surgical treatment of *Acanthamoeba* keratitis.

Or-18: **Epidemiological features and risk factors in *Acanthamoeba* keratitis: about six cases**

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Introduction: *Acanthamoeba* keratitis is a rare but serious ophthalmologic condition due to Acanthamoeba spp., a widespread in soil and water amoeba.
Purpose : To assess the epidemiological features and the risk factors in patients with Acanthamoeba keratitis.

Methods : We have retrospectively reviewed six cases of confirmed *Acanthamoeba* keratitis in the ophthalmology department of Charles Nicolle's hospital in Tunis, Tunisia between november 2012 and february 2017. Acanthamoeba were found either in corneal scraping and/or contact lenses and/or contact lens cases or storage solutions.
Results : The mean age at the moment of the diagnosis was 40.3 years (min 21, max 83 years). Sex ratio was 1. The average delay between first symptoms and diagnosis was 41.6 days (+/- 29.1 days).

We seeked for risk factors of Acanthamoeba keratitis and randomly matched our cases with 17 cases of bacterial keratitis randomised in the age, gender and clinical presentation. Contact lens wear was a statistically significant risk factor (p=0.002). Neither traumatism nor ocular surgery were statistically significant risk factors p=0.384 and 0.463 respectively.

All the patients received at least two anti parasitic drops including a biguanide (Picloxydine dichlorhydrate Vitabact®,and/or Polyhexamethylene biguanide PHMB) and a diamidine (hexamidine 0.1% Désomédine ®) from a period ranging from 2 to 4 months. 4 patients received fluconazole per os for 2 to 3 months. 1 case needed penetrating keratoplasty one year after the oncet of the keratitis.
Final visual outcome was poor with mean visual acuity of 2.01 (+/- 0.76) logMar. Mean follow up was 11 months years (+/- 8.07 months).

Conclusion : Acanthamoeba keratitis is a serious condition which can be vision-threatening. Contact lens wear is a major risk factor. Early diagnosis and appropriate therapy leads to a better visual outcome.

Or-19: Spectrum of microbial keratitis in the Gap Bon region of Tunisia

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PURPOSE: To review the epidemiology, risk factors, microbiologic spectrum, and treatment of microbial keratitis during a 5-year period at the University hospital of Nabeul in TUNISIA.

METHODS: Retrospective chart review in the 5-year interval, 2015 through 2016. Primary outcome measures included patient age at presentation, best-corrected visual acuity (BCVA), risk factors, culture and sensitivities, treatment, and complication occurrence.

RESULTS: A total of 28 eyes with microbial keratitis were identified. At least one risk factor was present in 20 (71.4%) cases, with the most common risk factors being preexisting ocular disease, contact lens wear, and a history of trauma. Gram-positive organisms represented 47.3%, gram-negative organisms 32.1%, fungal organisms 13.6%, and Acanthamoeba 6.4% of corneal isolates. Gram staining correctly identified the organism in 20 (71.4%) culture-positive cases, and Staphylococcus aureus was the most common isolate, followed by Pseudomonas aeruginosa. Average BCVA at resolution was 3/10. The perforation rate was 14%. Ten percent (10%) of cases underwent urgent penetrating keratoplasty.

CONCLUSIONS: Microbial keratitis remains a clinical challenge. MK remains a threat to vision and to the eye, but the majority of cases respond to prompt and appropriate antimicrobial therapy. When a causative organism is not identified in microbial keratitis, visual acuity is not as severely affected, and fewer severe complications occur.

O-20: *Acanthamoeba* keratitis. Report of 3 cases diagnosed in southern Tunisia

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Objective: To report three cases of *Acanthamoeba* keratitis caused by contact lenses.

Methods: We report three cases of *Acanthamoeba* keratitis diagnosed at the Ophthalmology department and laboratory of Parasitology of sfax Hospital, (Tunisia).

Results: Three immunocompetent contact lens-wearing female showed bilateral infectious keratitis. Trophozoit and cysts of Acanthamoeba were revealed by corneal scrapings in two patinets and in the contact lens solution in one patient. Bilateral penetrating keratoplasty was needed for one patient because of bilateral corneal perforation. In the other two cases, treatment led to scarring of lesions with moderate sequellar opacities.

Conclusion : *Acanthamoeba* keratitis causes a progressive ulcerative keratitis, which is not replying to the common antimicrobial therapy.The number of patients with This pathology increased dramatically in the last few decades, mostly in contact lens wearers.Treatment options for *Acanthamoeba* keratitis include medical and surgical but their prognosis is poor because of a significant delay in diagnosis and frequently a lack of effective medical management. Early diagnosis and treatment are required to effectively manage this condition.

Or-21: Investigation of the prevalence of *Acanthamoeba* keratitis in Turkey

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Objectives : Free-living amoeba, *Acanthamoeba* spp is reported to be the most common protozoan all over the world. The protozoan have been isolated throughout the world from soil, rivers, sewage, sea water, drinking water, swimming pools, air, contact lenses and eyewash solutions, which could be the source of infection. *Acanthamoeba* is well known as an opportunist protozoan parasite as well as causing infections in humans. There have been reports of serious clinical form inculiding sight-threatening amoebic keratitis (AK) in contact lens wearers.

In recent years, the incidence of *Acanthamoeba* keratitis has increased with related to the use of contact lenses. The aim of this study was to investigate the prevalence of *Acanthamoeba* keratitis in Turkey.

Methods: Totally 109 samples (sixty-three corneal scrapings and fourty six contact lenses and contact lens solutions) were obtained from patients admitted to the Corneal Outpatient Clinic, Ege University Medical Faculty, Department of Ophthalmology. The patients provided written informed consent and patient information form completed by the clinician. The samples were examined with microscopic examination (Giemsa and Calcofluor White) .Each samples were inoculated onto the center of 1.5 % non-nutrient agar (NNA) plate covered with a lawn 100 µl *Escherichia coli* bacterial suspension. DNA was extrated by corneal scrapings directly by using QIAamp DNA Mini Kit. A PCR targeting 18S rRNA gene (GenBank no: U07413) was used to amplify ~120 bp region using the Forward and Revers primers (newly designed). After the products had been run in 2% agarose gel, they were imaged under UV light by using the Vilber Lourmat device.

Results: *Acanthamoeba* spp. was found positive with NNA method in one a patient with keratitis. *Acanthamoeba* spp. DNA was detected in six (5.50 %) out of 109 samples.

Conclusion: *Acanthamoeba* spp are commonly present in nature and due to serious infections they pose great importance for human health. This study is significant through assessing the prevalence of *Acanthamoeba spp*. in suspicious corneal pathology, contact lenses and its solutions. By the use of PCR method, which is high in sensitivity and specificity, six patients were detected as positive and thus molecular methods have very important roles for the diagnosis of *Acanthamoeba* infection as other parasitic infections.

Keyword: *Acanthamoeba* spp., prevelence, keratitis, PCR
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Or-22: *Acanthamoeba*-Fusarium mixed keratitis in a Spanish contact lens user

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Free Living Amoebae of *Acanthamoeba* genus includes non-pathogenic and pathogenic strains that are currently classified in 20 different genotypes, T1-T20. We have evaluated the presence of *Acanthamoeba* strains in soil samples of Gran Canaria Island, Canary Islands, Spain. *Acanthamoeba* is able to cause an infection of the cornea mostly in contact lens wearers which if not diagnosed and treated early could end in blindness. Unfortunately, until now, there is not a fully effective therapeutic agent against *Acanthamoeba* keratitis. In the later years, our laboratory has successfully applied the application of intraocular voriconazole in cases of severe keratitis.

A corneal scrape and contact lens maintenance solution samples was provided to our laboratory in order to check for the presence of *Acanthamoeba*. After that, they were inoculated onto non-nutrient agar (NNA) plates and were checked for the presence of *Acanthamoeba*. We carried on the identification of *Acanthamoeba* strains using Page´s morphologic key and characterized at the genotype level by sequencing the DF3 region located in the 18S rDNA gene of *Acanthamoeba* as previously described. Moreover, fungi were observed in the samples and were characterized at the genus level as *Fusarium* spp.

The *Acanthamoeba* strain was checked for sensitivity to voriconazole and thus treatment of the patient using intraocular doses of this drug were started. Presence of acanthamoebae was checked each week at the corneal and aqueous humour levels. After a week of treatment, no viable amoebae were detected. However, patient eye health decreased gradually and fungal growth was not able to be stopped. After three months, several keratoplastias and antifungal combinations, the patient requested enucleation of the eye. Unfortunately, in the reported case even though *Acanthamoeba* was eliminated a coinfection with Fusarium was not stopped in time and ended up with this fatal end. Awareness should be raised regarding the possible coinfection cases such as the one described in this work.

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Or-23: Failure of molecular detection of keratitis inducing *Acanthamoeba* strains in diagnostics

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An otherwise healthy 49-year-old female patient presented with keratitis in both inflamed eyes. She was a contact lens wearer and had no history of a corneal trauma. In this case Acanthamoebae could be detected in our laboratory from the cornea abrasion and from the fluid of the contact lens storage cases microscopically by culture methods. Viable amoebae derived from a sample of the solution of a contact lenses storage case could be detected on agar plates seeded with *Enterobacter cloacae* and confirmed hereafter microscopically as *Acanthamoeba* sp. based on the morphological features.

A successful therapy could be provided consequently.

Several polymerase chain reaction (PCR) assays for the detection of single or multiple species of FLA are available and offer the benefit of being sensitive and fast. Therefore, a multiplex quantitative PCR for the simultaneous detection of *Balamuthia mandrillaris*, *Naegleria fowleri* and *Acanthamoeba* sp. developed by the Centers for Disease Control and Prevention (CDC) was applied to samples derived from a patient with keratitis of unknown genesis in the context of routine screening for FLA. The PCR did not detect nucleic acids of any FLA species.

A commercially offered PCR specific for *Acanthamoeba* sp. used with DNA isolated from the cultivated amoeba as a template also failed in the detection of this FLA.

However, by DNA sequencing this amoeba was proven to be a T4 genotype of *Acanthamoeba* sp..

Microscopic confirmation of *Acanthamoeba* sp. within the samples after culture requires well experienced professionals while the successful cultivation of Acanthamoebae is time consuming and depends on the right choice of culture conditions, e.g. media, food source and temperature. This example impressively shows the need for complementary procedures in addition to the PCR in diagnostic of FLA since false negative results might occur. While the morphological microscopical method led to the correct diagnosis, a multiplex PCR and a commercial Specie-specific PCR showed false negative results regarding *Acanthamoeba* sp.

And, it was the second case with such an outcome in 2016 in our laboratory…

Or-24: Organ lysates of crocodile possess antimicrobial and antitumor activities

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Abstract

Crocodiles live in unsanitary conditions, feed on rotten meat, are exposed to heavy metals such as arsenic, cadmium, cobalt, chromium, mercury, nickel, lead, selenium, endure high levels of radiation, are among the very few species to survive the catastrophic Cretaceous-Tertiary extinction event, and yet live up to a 100 years. Logically, we hypothesize that crocodiles have developed mechanisms to defend themselves from noxious agents. We recently tested this hypothesis and demonstrated that various organ lysates of crocodile exhibit powerful antimicrobial and antitumor activities by measuring lactate dehydrogenase as a marker for cell death as well as re-growth of cells. The discovery of antimicrobial and antitumor activities in the crocodile will stimulate research in finding therapeutic molecules from unusual sources, and has potential for the development of novel compound(s) that may also overcome current drug resistance. Nevertheless, intensive research in the next few years will be required to realize these expectations.

Or-25: *In vitro* activity of 1H-Phenalen-1-one derivatives against *Acanthamoeba castellanii* Neff and its mechanisms of cell death

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Acanthamoeba is an opportunistic pathogen which is the causal agent of a sight-threatening ulceration of the cornea known as Acanthamoeba keratitis (AK) and, more rarely, an infection of the central nervous system called "granulomatous amoebic encephalitis" (GAE). The symptoms of AK are non-specific, and so it can be misdiagnosed as a viral, bacterial, or fungal keratitis. Furthermore, current therapeutic measures against AK are arduous, and show limited efficacy against the cyst stage of *Acanthamoeba*. 1*H*-Phenalen-1-one (PH) containning compounds have been isolated from plants and fungi, where they play a crucial role in the defense mechanism of plants. Natural as well as substituted PHs exhibit a diverse range of biological activities against fungi, protozoan parasites or human cancer cells. New substituted PHs have been tested in this study.

Objectives & Methods: The activity of the PHs were tested *in vitro* against the trophozoite stage of *Acanthamoeba castellanii* Neff using Alamar Blue® reagent. For the evaluation of drug-induced cytotoxicity effects over murine macrophages was used a commercial kit based on the measurement of lactate dehydrogenase (LDH) activity released to the media. Assays to measure the mitochondrial membrane potential using JC-1, or the phosphatidylserine (PS) externalization using annexine v, were performed in order to determine the type of induced cell death after incubation with P. Also we evaluate the membrane permeability with SytoxGreen probe and the ATP level after treatment.

Results: These compounds showed a dose-dependent inhibition effect on the proliferation of the amoeba, with IC_{50} values up to 25 µM for the trophozoite stage. Moreover, some PH derivative induced externalization of PS as well as a decrease in the mitochondrial membrane potential in *Acanthamoeba*. In most cases ATP level and the cytoplasmic membrane of the parasites were not altered after the treatment with PHs.

Conclusions and significance of the work: In conclusion, substituted PHs were active against the *Acanthamoeba* strain tested in this study, and some of them could induce an apoptosis-*like* process on *Acanthamoeba castellanii* Neff, avoiding an unnecessary immune response, and considering PHs as a good compounds for further studies.

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Or-26: Chlorine dioxide induces the amoebicidal effect on pathogenic *Naegleria fowleri, Acanthamoeba castellanii* and *A. polyphaga*

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Pathogenic free-living amoebae, *Naegleria fowleri* and *Acanthamoeba* spp., are widely distributed in soil and water. *N. fowleri* and some species of *Acanthamoeba* can cause serious human infections such as primary amoebic meningoencephalitis (PAM) or granulomatous amoebic encephalitis (GAE), respectively. Recently, it has been increased on *N. fowleri* infection or *Acanthamoeba* keratitis (AK) from the wrong water supply facilities or contact lens wearers. Chlorine dioxide (ClO_2), yellow-green gas is a powerful disinfectant which is 2.5 and 500,000 times more effective than chlorine-based disinfectants and alcohol, respectively. In this study, we used to ClO_2 gas from a ready-to-use product called "Puristic". The Puristic is a product of tubing stick type without necessitating the ClO_2 gas generation on site. To examine the amoebicidal effect of ClO_2 gas against *N. fowleri*, *A. castellanii* and *A. polyphaga*, Amoebic trophozoite or cyst were exposed to ClO_2 gas (0.064ppmv/min) for 12-48 hr. Amoebae maintained for 12-48 hr without exposure to ClO_2 gas were used for the control groups. The viability and growth rates of amoebae were assessed by microscopic examination. The results showed that the viability of amoebae was effectively inhibited by treatment with ClO_2 gas, as which their viability were assessed by re-cultivation with each fresh medium. The growth rate of *N. fowleri* trophopzoite exposed with ClO_2 gas for 24 hr was completely inhibited. Whereas, the growth rates of *A. castellanii* and *A. polyphaga* trophozoite exposed to ClO_2 gas for 24 hr were decreased by 50 and 60% respectively. Furthermore, the actin mRNA levels of amoebae checked by RT-PCR were significantly reduced by treatment of ClO_2 gas. In case of cyst, the growth rates of *A. castellanii and A. polyphaga* were significantly inhibited when they were exposed ClO_2 gas for 12 hr. Taken together, ClO_2 gas induces amoebicidal effect on *N. fowleri, A. castellanii* and *A. polyphaga*. These results show that the treatment of chlorine dioxide may be the useful practice for the prevention and control of *N. fowleri* and *Acanthamoeba* contamination.

Or-27: Evaluation of the Anti-*Acanthamoeba* activity of two commercial eye drops commonly used to lower eye pressure

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Efficient treatments against *Acanthamoeba* Keratitis (AK), remains until the moment, as an issue to be solved due to the existence of a cyst stage which is highly resistant to most chemical and physical agents. In the present study, two antiglaucoma eye drops were tested for their activity against *Acanthamoeba*. The amoebicidal activity of the tested eye drops was evaluated against four strains of *Acanthamoeba* using Alamar blue method. For the most active drug the cysticidal activity against *A.* castellanii Neff cysts and further experiments studying changes in chromatin condensation levels, in the permeability of the plasmatic membrane, the mitochondrial membrane potential and the ATP levels in the treated amoebic strains were done. Even though both eye drops were active against the different tested strains of Acanthamoeba, statistical analysis revealed that one of them (Timolol Sandoz) was the most effective one against all the tested strains presenting IC_{50s} ranging from 0.529 % ± 0.206 for the CLC 16 strain to 3.962 % ± 0.150 for the type strain *Acanthamoeba castellanii* Neff. Timolol Sandoz 0.50% seems to induce amoebic cell death by damaging the amoebae at the mitochondrial level. Considering its effect, Timolol Sandoz 0.50% could be used in the case of contact lens wearers and patients with glaucoma.

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Or-28: Evaluation of Amoebicidal effects and cytotoxicity of multipurpose contact lens disinfecting solutions in Korea

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Main risk factors of *Acanthamoeba* keratitis are contact lens wear and their cleaning solutions. Most contact lens wearers use multipurpose contact lens disinfecting solutions (MPDS) for cleansing and disinfecting microorganism because of its convenience. We determined the amoebicidal effects of commercial MPDS in Korea and their cytotoxicity on human corneal epithelium cells. Fifteen MPDS made in Korea (A to O) and 5 kinds of imported MPDS (P to T) were tested for their amebicidal effect on *A. castellanii* trophozoites and cysts by using a most probable number (MPN) technique. Among them, 10 of korean MPDS showed little or no amoebicidal effect for 24 h exposure. A and B solutions showed 10% amoebicidal effect, and N and O solutions killed almost trophozoites and cysts within 24 h exposure. However, 50%-N solution showed 56% cytotoxicity on human corneal epithelial cells within 4 h exposure, and 50%-O solution also showed 62% cytotoxicity on human cells within 4 h exposure. Solution A did not show the cytotoxicity on human cells. All of the imported MPDS showed strong amoebicidal effect on trophozoites since 8 h exposure while they showed inadequate amoebicidal effect on cysts even 24 h exposure. Among them, P, Q, and T solution were tested for their cytotoxicity on human corneal epithelium cells. P and T solution showed 55.9% and 58.5% cytotoxicity on human cells with 4 h exposure at 70% concentration. Solution Q showed 61.6% cytotoxicity on human cells with 4 h exposure even 40% low concentration. These results revealed that most of MPDS made in Korea were ineffective to kill *Acanthamoeba* and the solutions which had amoebicidal activity showed high levels of cytotoxicity on human corneal epithelial cells. Five kinds of imported MPDS were ineffective to kill *Acanthamoeba* and most of them had cytotoxicity on human corneal epithelial cells. New formulation for improved MPDS, amebicidal but safe for host cells, are needed to be developed to prevent *Acanthamoeba* keratitis in Korea.

Or-29- Status of the effectiveness of contact lens solutions against keratitis-causing pathogens

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Purpose: The aim of this study was to assess the antimicrobial effects of contact lens disinfecting solutions in Malaysia and to determine whether targeting cyst wall would improve the efficacy of contact lens disinfectants.

Methods: Using ISO 14729 Stand-Alone Test for disinfecting solutions, bactericidal and amoebicidal assays of six different contact lens solutions including: ReNu Multipurpose, FreshKon Clear, Opti-Free Multipurpose, Complete Revitalens, Oxysept Multipurpose, AO-sept Multipurpose were performed. The efficacy of contact lens solutions was determined against keratitis-causing microbes, namely: *Pseudomonas aeruginosa*, Methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Acanthamoeba castellanii*. In addition, using chlorhexidine as an antiamoebic compound and cellulase enzyme to disrupt cyst wall structure, we determined whether combination of both agents can enhance efficacy of marketed contact lens disinfectants against *A. castellanii* trophozoites and cysts, in vitro.

Results: The results revealed that all contact lens disinfectants tested showed potent bactericidal effects exhibiting 100% kill against all bacterial species tested. However, none of the contact lens disinfectants had any effect on *Acanthamoeba* cysts viability. When tested against trophozoites, two disinfectants, Oxysept Multipurpose and AO-sept Multipurpose showed partial amoebicidal effects. Using chlorhexidine as an antiamoebic compound and cellulase enzyme to disrupt cyst wall structure, the findings revealed that combination of both agents in the contact lens disinfectants abolished viability of *A. castellanii* cysts and trophozoites.

Conclusions: Given the inefficacy of contact lens disinfection solutions tested in this study, these findings present a significant concern to the public health. These findings revealed that targeting cyst wall by using cyst wall degrading molecules in contact lens disinfecting solutions will enhance their efficacy against this devastating eye infection.

Or-30: Human Innate Immunity and Acanthamoeba: effect of oral hormonal contraception

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PURPOSE. Recently, we have characterize the pattern of pro-inflammatory and anti-inflammatory cytokins released by human peripheral blood mononuclear cells (PBMCs) and human monocyte derived macrophages (MDMs) in response to *Acanthamoeba*. It is known that sex hormones can strongly regulate immune responses and influence homeostasis of eye and brain, privileged sites of *Acanthamoeba* infections. Considering the widespread use of oral contraceptives (OCs) and noting that monocytes and macrophages express receptors for sex hormones, the aim of this study was to evaluate whether the use of OCs can affect monocyte and macrophage activity against *Acanthamoeba*. To this end, we evaluate the effect of OCs on pro-inflammatory and anti-inflammatory cytokines release by PBMCs and MDMs in response to *Acanthamoeba*-derived cell-free conditioned medium (aCM) and to known agonists of TLR4 and TLR2 receptors (used as positive controls).

METHODS. PBMCs from healthy adult women treated (FOCs) or non-treated (Fs) with OCs were isolated during the follicular phase of their menstrual cycle. MDMs were obtained from each sub-population, and characterized by evaluating the decrease in the surface marker CD14. Then all samples were stimulated with either aCM, LPS or Pam2CSK4, *in vitro*. Release of TNF-α, IL-6, IL-10 and IL-8 was investigated at specific hours post-stimulation, by Enzyme Linked Immuno Sorbent Assay (ELISA). All experiments were performed in triplicate. Statistical difference was evaluated with the two-tailed Student *t* test and two-way analysis of variance (ANOVA), followed by Bonferroni post test, using Graph-Pad Prism (GraphPad Software Inc.).

RESULTS. Of interest, our data showed that, the use of OCs affected mainly on PBMCs aCM-stimulated, significantly reducing the production of all the cytokines studied. Whereas, only a highly significant reduction of IL-8 and IL-10 was detected in FOC-MDMs in comparison with F-MDMs.

DISCUSSION. At the moment it is difficult to explain the impact that OCs assumption might have in *Acanthamoeba* infections, in that it reduce the production of both pro-inflammatory and anti-inflammatory cytokins. However, considering the peculiar ability of *Acanthamoeba* cause premature release of IL-10 by monocytes/macrophages, we hypothesize that its lower production, by both FOC-PBMCs and FOC-MDMs, might promote the inflammatory response and monocyte/macrophage antimicrobial activity, to the detriment of the protozoan.

CONCLUSIONS. For the first time, it is demonstrated that, during the early phase of infection, the use of OCs might interfere in the mechanisms that occur between monocytes/macrophages and *Acanthamoeba*.

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Or 31: Qualitative analysis of human monocyte and macrophage proteomes after stimulation with Acanthamoeba soluble products: a preliminary study

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Acanthamoeba castellanii is a free-living amoeba ubiquitous in nature, with a worldwide distribution. Despite its free-living mode of life, *Acanthamoeba* can act as facultative parasite causing severe chronic infectious diseases mainly involving immune-privileged sites such as the brain and the eyes. Dissecting host innate immune system and *Acanthamoeba* interactions might suggest interesting insights for the development of new therapeutic regimes, addressed to ameliorate the inflammation at the site of infection while eradicating the pathogen. So far, immunological and molecular techniques have been applied to understand the mechanisms at the interface between innate immune cells and *Acanthamoeba*, however the proteomics approach has not been performed yet.

OBJECTIVES: All this considered, the aim of the study was to characterise the proteome of human primary monocyte and macrophages after challenge with *Acanthamoeba* in order to understand if *Acanthamoeba* might modulate protein expression in these innate immune cells and the pathways are mainly involved.

METHODS: Towards this purpose, the total proteome of human peripheral blood derived monocytes and human monocyte-derive macrophages, stimulated with *Acanthamoeba* (Genotype T4) conditioned medium (aCM), was extracted and subsequently digested for shotgun gel-free proteomic analysis through LC/MS-MS techniques.

RESULTS: 3669 proteins were identified in human monocyte samples, of which 75 and 83 were found majorly expressed in un-stimulated and aCM stimulated monocytes, respectively ($P<0.05$, $-1\leq Rsc \geq 1$). 4884 proteins were identified in human macrophage samples, of which 92 and 57 were found majorly expressed in un-stimulated and aCM-stimulated macrophages, respectively ($P<0.05$, $-1\leq Rsc \geq 1$). Pathway analysis showed that *Acanthamoeba* soluble products modulated the expression of proteins involved in cell adhesion, integrin signaling pathway, inflammation mediated by chemokine and cytokine signaling pathway, antigen processing and presenting and plasminogen activating cascade in monocytes. In macrophages, stimulation with aCM modulated the expression of protein involved in complement and coagulation cascade.

CONCLUSION: Overall our study provide preliminary insights into the mechanisms by which *Acanthamoeba* may influence protein expression in monocyte and macrophages and consequently their state of activation and the outcome of infections. The use of this newest techniques in the study of host/*Acanthamoeba* interactions could have practical use in pharmaceutical and diagnostic fields.

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Or-32: **Detection of serum antibodies in children and adolescents against *Balamuthia mandrillaris*, *Naegleria fowleri* and *Acanthamoeba* T4**

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The presence of free-living amoebae of the genera *Naegleria, Acanthamoeba* and *Balamuthia,* which contain pathogenic species for humans and animals, has been demonstrated several times and in different natural aquatic environments in southern Sonora, Mexico. Likewise, 500 serum samples from healthy male adults, who attended to donate blood, were assayed for antibodies against *N. fowleri* and results showed a high prevalence of IgG antibodies, indicating exposure to the ameba, but sera did not recognize the same antigen proteins, concluding that the differences in proteins recognition could be due to other *Naegleria* species. With the aim of continue adding information about the immunology of pathogenic free-living amoebae, 117 sera from children and adolescents living in three Yaqui towns, with a semi-arid climate with poor humidity most of the year and an intensive agricultural system. Humoral IgG response against *B. mandrillaris*, *N. fowleri* and *Acanthamoeba* T4, were analyzed in duplicate to titers 1:100 and 1:500 by enzyme-linked immunosorbent assays (ELISA). The cut-off was calculated as the mean of the negative controls + 3 standard deviations. For *Balamuthia,* complete amoebas and lysed were tested as antigens. Children and adolescents were between 5 and 16 years old, with a mean of 9 years and 55% were males.

As results for *Balamuthia* we can anticipate that the optical density readings were higher when whole cells were used as antigens, instead of lysed cells. Clearly, three types of sera are distinguished: sera with high titers against *B. mandrillaris*, sera with high titers against *N. fowleri* and sera with high titers against both amoebae. It remains only to compare the results of sera against *Acanthamoeba*, to have better information on the specificity or not of sera against these three amoebae studied. The sera with higher specific and non-specific titers will be selected to continue the proteomic studies of the surface antigens of *Balamuthia mandrillaris*, which could be used for diagnostic purposes.

Or-33: ***Balamuthia mandrillaris* coexisting with other thermophilic amoebae in a natural source of thermal water**

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Little is known about the prevalence of *Balamuthia mandrillaris* in the environment due to its difficulty of the isolation, but once stablished the axenic culture is relatively easy maintaining it. Since most of the time the researchers are interested primarily in isolate *B. mandrillaris* from environmental samples, the accompanying flora becomes second place in importance. Thus, this study was aimed at determining which potentially pathogenic free-living amoebae and *B. mandrillaris*, can be found cohabitating in a source of natural thermal water. A place called "Agua Caliente" (Hot water), where people use to visit and introduce their feet with curative purposes, was sampled twice, in March 2015 and July 2016, searching for *B. mandrillaris*, *N. fowleri* and *Acanthamoeba* spp. In the first sampling 500ml of water were concentrated by centrifugation and DNA was extracted to run a PCR using specific primers for *N. fowleri* and *B. mandrillaris*. In the second sampling the procedure to obtain DNA direct from the water sample was repeated, and also another 50 mL were concentrated and sediment was seeded on non-nutritive agar plates added with *Escherichia coli*, to intent the isolation of *B. mandrillaris* and other free-living amoebae using the isolation protocols described for the three genera mentioned. In both dates of sampling, the presence of *B. mandrillaris* was positive for the PCR technique on the direct DNA extracted from water, but we failed in to make it growth on culture media. 17 strains of thermophilic amoebae were isolated after incubation at 45°C, and 21 Other amoebae using 37°C as isolation temperature. Identifications are running of the PCR products obtained with primers ITS1 and ITS2 for *Naegleria* species, JDP1 and JDP2 for *Acanthamoeba* genotypes and ERIB1 and ERIB10 for other amoebae. The presence of *B. mandrillaris* in this kind of water used for medical purposes according to the popular believing, the free-living amoeba biodiversity and the temperature of water, will be discussed and will give more information about the niche and potential risk of *B. mandrillaris*.

Or-34: Mitochondrial genome of *Acanthamoeba* and the relevance of genotype assignment

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All extant eukaryotes possess mitochondria or their related organelles that are vital for cellular survival. Mitochondria play a central role in energy transduction, ion homeostasis, intermediary metabolism and apoptosis. Mitochondria contain genomes involving in respiration and/or oxidative phosphorylation, translation, transcription, RNA maturation and protein import. Like most other eukaryotes, the mitochondrial genome architecture of acanthamoebae exhibits circular supercoiled DNA with size variation among strains/genotypes, ranging from 38.7 to 44.0 kb. The genes identified in *Acanthamoeba* mitochondrial genome encode large and small subunit ribosomal RNAs, transfer RNAs, large and small subunit ribosomal proteins, enzymes in electron transport chain and ATP synthase complex, and several unknown open reading frames. Besides being biological importance, the cytochrome *b* locus of mitochondrial genome has been deployed to differentiate some apicomplexan protists such as all 6 species of *Plasmodium* causing human malaria. Although the nuclear small subunit ribosomal RNA gene has been considered to be useful for genotyping of the genus *Acanthamoeba*, ambiguity in genotype assignment due to indels in sequence alignment has been occasionally encountered. Because accurate genotyping of *acanthamoebae* has clinical and taxonomical importance, in this report we have determined the mitochondrial apocytochrome b (*cob*) sequences of 10 genotypes including subtypes in genotype T4 of *Acanthamoeba* from Thai isolates derived from environmental and clinical samples. Phylogenetic construction has shown that the genotypes inferred from the *cob* sequences gave similar results with that determined from the nuclear small subunit ribosomal RNA gene. It is noteworthy that subtypes within the T4 genotype could also be differentiated based on the *cob* sequences. Therefore, the mitochondrial *cob* locus is a promising alternative gene target for genotypic determination of *Acanthamoeba*.

Or-35: **Analysis of the nuclear small subunit ribosomal RNA gene of *Acanthamoeba* and identification of two novel genotypes in Thailand**

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Genotyping of the genus *Acanthamoeba* has important taxonomical and clinical relevance because certain genotypes have been incriminated in human morbidity and mortality. The prevalence and distribution of *Acanthamoeba* genotypes from natural water sources and fish gills in Thailand have been previously presented. Although sequencing of the diagnostic fragment 3 (DF3) of the 18S rRNA (*Rns*) gene have been applied for genotyping of *Acanthamoeba*, some genotypes (e.g. T18 and T19) could be misidentified based on these short sequences. In the present report, we have determined the genotypes of acanthamoebae spanning >2 kb from 272 isolates (68 from natural water sources and 204 from fish gills). In total, 12 genotypes were identified, i.e. T2/6b, T3, T4, T5, T11, T12, T13, T17, T18, T20 including 2 new genotypes. Genotype T4 was most common among isolates from water sources and fish gills. Analysis of subtypes in genotype T4 has shown that isolates from natural water sources could be classified as T4B, T4C and T4F whereas all subtypes except T4E occurred among isolates from fish gills. Although a novel genotype contained DF3 sequence belonging to genotype T9, a number of nucleotide substitutions occurred at the 5' portion encompassing DF1 and DF2 regions. The other novel genotype was closely related with genotype T14 but exhibited >8% sequence dissimilarity. It is noteworthy that novel genotypes seem to be rare in nature while identification of these genotypes required sequences spanning >2 kb of the *Rns* locus.

Or-36: Evidence of a M1-Muscarinic GPCR homolog in unicellular eukaryotes: featuring *Acanthamoeba* spp. Bioinformatics 3D-modelling and experimentations

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Background: Acetylcholine affects the target cellular function via muscarinic and nicotinic cholinergic receptors that are seen to exist in humans. Both the cholinergic receptors are G-Protein coupled receptors (GPCRs) that perform cardinal functions in humans. Anti-muscarinic drugs, particularly the ones that target M1 subtype (mAChR1), have consistently shown to kill unicellular pathogenic eukaryotes like *Acanthamoeba* spp (1).

Objective: As the M1 receptor subtype has not been reported to be expressed in the above protist, the presence of an ancient form of the M1muscarinic receptor was inferred (2). Bioinformatic tools and experimental assays were performed to find the expression of ligand binding site.

Methods: A search for sequence homology of amino acids of human M1 receptor failed to uncover an equivalent ligand-binding site on *Acanthamoeba*, but structural bioinformatics showed a hypothetical protein L8HIA6 to be a structural homolog of the human mAChR1.

Results: Immunostaining with an anti-mAChR1 antibody showed cellular staining. Growth assays showed proliferation and lethal effects of exposure to mAChR1 agonist and antagonist respectively. With the recent authentication of human mAChR1 structure and its addition to the database, it was possible to discover its structural analog in *Acanthamoeba*; which explains the effects of anticholinergics observed in the past on *Acanthamoeba* spp.

Conclusion: With a narrow choice of drugs to treat fatal meningoencephalitis caused by *Acanthamoeba* spp, the discovery of an ancestral mAChR1 on *Acanthamoeba* could prove to be a potential therapeutic target in the future.

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Or-37: The Status of Molecular Analyses of *Acanthamoeba* Isolates Maintained by International Culture Collections.

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The genus *Acanthamoeba* represents one of the most ubiquitous protistan groups in nature. Members occupy a variety of ecological niches, ranging from soil to water, as well as in dust and in the atmosphere, while geographically *Acanthamoeba* can be isolated from the tropics to almost polar conditions. Our knowledge of the biological diversity of *Acanthamoeba* comes in part from the use of standard strains maintained by the major microbial culture collections, ATCC (including BEI Resources) and CCAP. The use of standard strains is vital for ensuring the comparability of research. The diversity of standard strains of Acanthamoeba is reviewed, emphasizing the status of genotypic studies of these strains by DNA sequencing of the small subunit ribosomal RNA of either the nucleus (18S rRNA gene; *Rns*) or the mitochondria (16S-like rRNA gene; *rns*). Over 170 different strains have been maintained by the culture centers, and DNA sequence information is available for more than 70% of these strains. Determination of the genotypic classification of standard strains within the genus indicates that the frequencies of types only roughly mirror that seen in strains from clinical or environmental studies, showing significant differences in the frequency of some genotypes. The standard strains include the type isolate from almost all named species of *Acanthamoeba*, allowing an evaluation of the validity of species designations. Multiple species share the same sequence type, while multiple sequence types have been identified for different strains that share the same species name. Some questions are raised concerning the issue of sequence reliability. Comparison of information from the sequences of *Rna* and *rns* genes from *Acanthamoeba* genomes deposited in the DNA databases, and from comparisons of sequences from different laboratories raises a significant possibility that some standard strains have been mislabeled when studied, leading to potential problems for comparative analyses. It is important that all species have reliable genotype designations. In the future, the culture collections should be encouraged to assist in completing the molecular inventory of standard strains, while workers in the *Acanthamoeba* research community should endeavor to ensure that strains representative of genotypes that are missing from the culture collection are provided to the culture centers for preservation.

Or-38: *Mycobacterium llatzerense*, a new amoebae resisting bacteria found in drinking water.

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Nontuberculous mycobacteria (NTM) are increasingly associated to public health problems. It has been shown that these bacteria, among others, are able to resist phagocytosis via various mechanisms. In the environment, free-living amoebae (FLA) feed on bacteria by phagocytosis, but several bacteria are resistant to this predation. Thus, free-living amoebae can be seen as a training ground for pathogenic bacteria. We have previously described *Mycobacterium llatzerense* as the major NTM associated to FLA in a drinking water network.

In this study, our objective was to analyze the interactions at the molecular and cellular level between *Mycobacterium llatzerense* and FLA.

M. llatzerense was highly resistant to *A. castellanii* predation on plate. In addition, they survived and multiplied in a liquid co-culture with *A. castellanii*. Microscopic studies showed that *M. llatzerense* was phagocytosed and frequently possess lipidic inclusions, suggesting a subversion of host resources. Using a genomic-based comparative approach, twelve genes involved in phagocytosis interference, shared with *M. tuberculosis*, were identified in the *M. llatzerense* genome, sequenced in this study. Transcriptomic analyses showed that ten of these genes were significantly upregulated during the first hours of the infection, which could partly explain its resistance. Finally, *M. llatzerense* was shown to actively inhibit phagosome acidification.

In conclusion, *M. llatzerense* presents a high degree of resistance to FLA phagocytosis likely explaining its high occurrence within FLA isolated from a drinking water network. It underscores that NTM might be carefully followed in these networks to anticipate future health concerns.

Or-39*: Legionella pneumophila* prevents proliferation of *Acanthamoeba castellanii*

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Acanthamoeba castellanii is considered as a "trojan horse" of the microbial world as it allows multiplication and protects several bacteria. *Legionella pneumophila*, the causative agent for legionellosis, is one of the most studied *A. castellanii* resisting bacteria. Many host cellular pathways are modulated by *L. pneumophila* such as phagocytosis and autophagy.

Our objective was to test whether *L. pneumophila* would modulate the proliferation of *A. castellanii.*

We found that *L. pneumophila* is able to impair proliferation of infected *A. castellanii*. This effect seemed controlled by an effector secreted by *L. pneumophila* since a mutant in its type-IV secretion did not impair proliferation. Time lapse microscopy showed that, in addition to impair cell division, *L. pneumophila* induced modifications in shape, motility of *A. castellanii.* Use of Edu, an analogue of thymidine, demonstrated that infection inhibited DNA replication within *A. castellanii.* Thus, we searched for cyclin dependent kinase (CDK) genes in the *A. castellanii* genome and found one gene, *CDC2b*, which is similar to the main cell cycle regulator gene in human *CDK1*. By genetic complementation experiments, we establish that the amoebal protein CDC2b could be a cyclin-dependent kinase (CDK). To our knowledge, *L. pneumophila* could be the first bacterium regulating the eukaryotic cell cycle through down-regulation of the host CDK expression. In conclusion, *L. pneumophila* impairs *Acanthamoeba castellanii* cell cycle by a mechanism which remains to be elucidated.

Or-40: Description of the early events of *Acanthamoeba culbertsoni* invasion in the model of granulomatous amoebic encephalitis (GAE) in diabetic mice

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Granulomatous amoebic encephalitis (GAE) is a chronic, difficult to resolve infection caused by amphizoic amoebae of the genus *Acanthamoeba*, which in most cases occurs in immunosuppressed persons or with chronic diseases such as diabetes.

In this work we describe the early events of *A. culbertsoni* (T4) infection of GAE model in diabetic mice. The strain in study was isolated from a case of amebic keratitis with extra-corneal invasion. Diabetes was induced in 12 male BALB /c mice (7 weeks old), with a single intraperitoneal dose of streptozotocin (130 mg/kg). Once established diabetes (6 weeks post-induction), $1x10^6$ trophozoites were inoculated via intranasal. Then were sacrificed and fixed by perfusion at 24, 48, 72 and 96 h post-inoculation, the brains and nasopharyngeal meatus were processed according to the immunohistochemical technique.

Analysis of the results showed that since the earlier time evaluated (24 h), trophozoites and scarce cysts were immunolocalized in the respiratory epithelial bone tissue, olfactory nerve packages, Schwann cells, and the epineurium base. After 48 h, trophozoites were observed in the respiratory epithelium, white matter of the brain, subcortical central cortex and nasopharyngeal associated lymphoid tissue (NALT). At 72 h, cysts and trophozoites were immunolocalized in the olfactory bulb with the presence of a low inflammatory infiltrate characterized by polymorphonuclear cells. Scarce amoebae were observed in the granular layer of the cerebellum without evidence of inflammation or tissue damage. Otherwise in the evaluated tissues no amoebae were observed at 96 h. It is important to highlight that no evidence of destruction, either inflammatory infiltrate in the surrounding tissues where the amoebas were immunolocalized, which could contribute to the rapid spread of infection, particularly in diabetic mice.

Invasion of trophozoites in diabetic mice is significantly greater with respect to healthy mice of the same age inoculated under the same conditions, suggesting that diabetic mice are more susceptible to GAE. The data obtained suggest that trophozoites invade the tissues by separating the superficial cells, penetrating between the junctions without causing cytolytic effect in the adjacent cells and later reaching the CNS.

Or-41: First Report of *Vermamoeba vermiformis* In Clinical Samples From Patients In Venezuela

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Vermamoeba vermiformis, a free-living amoeba, has been involved in public health, being reported as a possible causative agent of meningoencephalitis, bronchoneumonía and keratitis, as well as a pathogen bacteria carrier. In this sense, *V. vermiformis* has been isolated alone or co-infecting along with other eye pathogens as *Acanthamoeba.* In Venezuela, there are no reports about infections caused by or related to *V. vermiformis*. At Laboratory of Amoebiasis-Central University of Venezuela, two (2) *V. vermiformis* isolates were obtained from patients who suffered corneal ulcers. One of the patient was a contact lenses user and had a mixed infection with *Acanthamoeba* genotype T4 and *V. vermiformis.* *V. vermiformis* was isolated both in the eye and the contact lenses, meanwhile *Acanthamoeba* was only recovered from the eye sample. The other patient was not a contact lenses wearer and *V. vermiformis* was obtained from the lesion. The diagnosis was achieved by culture and polymerase chain reaction at the University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, Tenerife-Canary Islands-Spain. This is the first time the presence of *Vermamoeba vermiformis* in clinical cases is described in Venezuela and is the first report of a mixed infection with *Acanthamoeba* and *Vermamoeba vermiformis* in this country.

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Or-42: *Acanthamoeba castellanii* is a potential host for *Streptococcus pyogenes* and *Streptococcus pneumonia*

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Among the genus *Streptococcus*, *S. pyogenes* and *S. pneumoniae* are the major causes of pharyngitis, impetigo, pneumonia and meningitis in humans. *Streptococcus* spp. are facultative anaerobes that are nutritionally fastidious, yet survive in the environment and target the predisposed population. Antibacterial disinfectants have been partially effective only, indicating the need for novel preventative measures and to understand mechanisms of bacterial resistance. *Acanthamoeba* is a free-living protist that is known to harbour microbial pathogens, provide shelter, and assist in their transmission to susceptible population. The overall aim of this study was to determine whether *S. pyogenes* and *S. pneumoniae* can interact with *A. castellanii* by associating, invading, and surviving inside trophozoites and cysts. It was observed that both *S. pyogenes* and *S. pneumoniae* were able to associate as well as invade and/or taken up by the phagocytic *A. castellanii* trophozoite. Notably, *S. pyogenes* and *S. pneumoniae* survived encystation process, avoided phagocytosis, multiplied, and exhibited higher recovery from the mature cysts, compared with the trophozoite stage (approximately 2 bacteria per amoebae ratio for cyst stage *versus* 0.02 bacteria per amoeba ration for trophozoite stage). As *Acanthamoeba* cysts are resilient and can disperse through the air, *A. castellanii* can act as a vector in providing shelter, facilitating growth and possibly genetic exchanges. In addition, these interactions may contribute to *S. pyogenes* and *S. pneumoniae* survival in harsh environments, and transmission to susceptible population and possibly affecting their virulence. Future studies will determine the molecular mechanisms associated with *Acanthamoeba* interactions with *Streptococcus* and the evolution of pathogenic bacteria and in turn expedite discovery of novel therapeutic and/or preventative measures.

Or-43: Amoebicidal activity of α-bisabolol against the trophozoite stage of *Acanthamoeba castellani* Neff

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Abstract

Acanthamoeba genus includes opportunistic pathogens which are distributed worldwide and are causative agents of a fatal encephalitis and severe keratitis in humans and other animals. Until present there are not fully effective therapeutic agents against this pathogen and thus the need to search for novel anti-amoebic compounds is urgent. Recently, essential oils of aromatic and medicinal plants have shown activity against *Acanthamoeba* strains. Therefore, this study was aimed to evaluate the activity of main component of chamomile essential oil (a sesquiterpene) namely α-bisabolol against the *Acanthamoeba castellani* Neff strain. After evaluation of the activity and toxicity of this molecule, IC_{50} values of 20.839± 2.015 for treated amoebae as well as low cytotoxicity levels in a murine macrophage cell line was observed. Moreover, in order to elucidate mechanism of action of this molecule, changes in chromatin condensation levels, permeability of the plasmatic membrane, the mitochondrial membrane potential and the ATP levels in the treated amoebic strains were checked. The obtained results revealed that α-bisabolol was able to induce apoptosis, increase the permeability of the plasmatic membrane and decrease both mitochondrial and ATP levels in the treated amoebae. Therefore, and given the obtained results, α-bisabolol could be used a future therapeutic agent against *Acanthamoeba* infections.

Keywords

Acanthamoeba spp., *Matricaria*, essential oil, activity, α-bisabolol

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Or-44: **Antiparasitic activity of seaweed extracts from the Tunisian coast**

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The metabolic and physiologic ability of marine organisms, especially seaweeds, in dealing with highly complex and hostile environments have resulted in the evolution of a number of secondary metabolic pathways which have high prospects of yielding natural products with unique chemical structures. In this regard, different natural products isolated from marine organisms, mainly from seaweeds, have been reported to possess a broad spectrum of pharmacological properties, such as antiviral antiprotozoal, antibacterial, antioxidant, antifungal, cytotoxic and antitumoral activities. The success in the area of bioactive marine natural products has been already proven by the increasing number of new compounds in pre- or clinical evaluation, which are of interest from the point of view of potential drug development.

Free-living amoebae of genus *Acanthamoeba* are opportunistic pathogens widely distributed in the environment, and are the causative agents of several humans infections in, such as *Acanthamoeba* keratitis, the usually fatal granulomatous amoebic encephalitis and also disseminated infections. The existence of the cyst stage during the life cycle of this parasite complicates Acanthamoeba therapy as it is highly resistant to antibiotics and physical agents. All these facts reinforced the necessity to find and develop an effective therapy against *Acanthamoeba* infections.

In the present study, we are interested to some seaweeds species collected from the Tunisian coasts and belonging to the 3 types (brown, green and red alga). The aim was to test different organic extract and to identify eventual antiprotozoal activity against amoeba in order to isolate actives molecules and elucidate their action mode on *Acanthamoeba* genus and especially *A. castellani* neff. This research showed promising results through an interesting activity of a red alga.

Acknowledgments: This work was supported by the RICET grants (project RD12/0018/0012 of the programme of Redes Temáticas de Investigación Cooperativa, FIS), Spanish Ministry of Health, Madrid, Spain; and Project BIO24 "Principios activos inductores de apoptosis en la quimioterapia de tripanosomosis y leishmaniosis" (project 2016_25) from Obra Social La Caixa-Fundación CajaCanarias. ALA and IS were funded by the Agustín de Betancourt Programme.

Or-45: *In vitro* study of *Acanthamoeba culbertsoni* isolated from a clinical case with intraocular dissemination

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Acanthamoeba spp., includes species of free-living amoebae identified as human pathogens and can cause dangerous human illness such as chronic granulomatous amoebic encephalitis (GAE) and amoebic keratitis, a sight-threatening infection of the cornea correlated to contact lens mistreatment. Acanthamoebae used in this study were isolated from an extra-corneal case of keratitis. Amoebae migrated throughout the diverse layers of the cornea producing dissemination of trophozoites reaching the aqueous humor.

In this study, we carried out assays *in vitro* concerning the general morphological characterization and some biological features as well as interactions with hamster cornea and MDCK epithelial cells monolayers of *Acanthamoeba culbertsoni*.

Amoebae were grown in axenic medium (Bactocasitone), enriched with 10% fetal bovine serum. Optical, transmission and scanning electron microscopy were used as well as measurements of transepithelial electrical resistance (TER). Interactions were carried out on MDCK epithelial cells monolayers and hamster cornea.

The analysis of results indicates that this amoeba is invasive because since 2 h of interaction, trophozoites penetrated the most superficial layers of corneal epithelial layers, reaching the stroma after 12 h. It is important to highlight that comparing these results with those obtained in previous assays, this is the first time that we observed amoebae migrating and penetrating to the corneal stroma, since other *Acanthamoeba* strains, only penetrate to the basal cells of the cornea after 24 h of interaction. These observations correlated with TER readings and the presence of ruthenium red used as an intercellular tracer.

The *in vitro* results may correlate the observations reported in the clinical case concerning the penetration of this amoeba to deeper layers of hamster cornea.

Or-46: **In vivo CNS infection model of Acanthamoeba genotype T4: the early stages of infection**

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Acanthamoeba genus has a wide global distribution, as they can be isolated from diverse environments: bodies of water, soil and even from the air in cysts form.

Acanthamoeba genus is the etiological agent of skin lesions, keratitis and granulomatous amoebic encephalitis. At present, the complete understanding of the pathogenesis and pathophysiology of *Acanthamoeba* encephalitis it is unknown, especially those events occurring during the initial stages of infection.

This study was carried out in order to describe the early morphological events observed during the invasion of two pathogenic strains of *Acanthamoeba* (genotype T4); *A. castellanii* and *A. culbertsoni*, at the olfactory meatus and cerebral, pulmonary, renal, hepatic and splenic tissues, in an *in vivo* GAE murine model. The histological and immunohistochemical description of the events at 24, 48, 72 and 96 h post intranasal inoculation of BALB/c mice were performed. *A. castellanii* shown higher invasion rate than *A. culbertsoni*. The present study supports previous evidences of lack of inflammatory response during the early stages of infection. *Acanthamoeba* invasion through CNS and different organs evaluated is a slow and contact dependent process. The early morphological events during the invasion of amoebae consisted of trophozoites penetration to different epithelia; olfactory, respiratory, alveolar space and renal tubule, which resemble the process of amoebae invasion described in corneal tissue. Through these results, it is possible to describe that trophozoites after reaching the nasal epithelium continued their invasion separating and lifting the most superficial cells then migrate and penetrate between the cell junctions without causing a cytolytic effect on adjacent cells. These results confirm the idea that dependent mechanisms of contact are relevant for amoebae of the genus *Acanthamoeba* regardless of the invasion site.

Or-47: Evidence Targets of Aminodarone in *Acanthamoeba* spp. Bioinformatics and experimentations.

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Background: Ion channels coupled to cell membrane receptors are seen in many multicellular eukaryotes to execute several metabolic and survival functions. Their role in the biology of the unicellular pathogenic eukaryotes, like *Naegleria fowleri* and *Acanthamoeba* specie has emerged recently (1).

Objective: Amiodarone, that binds potassium and calcium ion channels is being reported to be amoebicidal in pathogenic eukaryotes (2); therefore we hypothesized the presence of a homologous targets in *Acanthamoeba* spp.

Methods: Methodologies used were, bioinformatics analysis, structure activity relationship, ligand binding prediction and growth assays.

Results: We show the evidence for the presence of homology in targets of Amiodarone in humans with similar targets in *Acanthamoeba*. Growth assays with Amiodarone showed growth inhibitory effects.
Conclusion: Exploration of the targets of Amiodarone is expected to not only design better drugs for the treatment of diseases caused by *Acanthamoeba* but also would improve our understanding of the evolution of ion channels coupled to receptors in *Acanthamoeba* spp.

Keywords: *Acanthamoeba*, Amiodarone, Protein homology

1. **Baig AM, Ahmad HR.** Evidence of a M1-Muscarinic GPCR homolog in unicellular eukaryotes: featuring Acanthamoeba spp bioinformatics 3D-modelling and experimentations. *J Recept Signal Transduct Res*. 2016 Sep 7:1-9.
2. **Baig AM, Iqbal J, Khan NA.** In vitro efficacies of clinically available drugs against the growth and viability of an Acanthamoeba castellanii keratitis isolate belonging to the T4 genotype. Antimicrob Agents Chemother 2013;57:3561-3567.

POSTER PRESENTATION

P-1: Primary Amoebic Encephalitis Preventive Nose Plugs: Prophylaxis against *Naegleria fowleri* Infection

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Objective: Naegleria fowleri is a free-living amoeba; it is a protist pathogen that is known to cause a fatal encephalitis in humans known as "Primary Amoebic. Mostly, PAM is reported in recent swimmers and people who perform ablution and nasal cleansing. Much has been done for vaccination and treatment without any success in past 60 years. One of the most alarming aspects of PAM is the mortality rate that has remained greater than 99% (1) in past 60 years Despite our advances in anti-amoebic chemotherapy, the treatment modalities are mostly empirical, which often results in deaths (2). Some drug delivery and diagnostic devices (3) have been proposed recently, but they are yet to be implemented . We propose a prophylaxis for this disease by introducing a device "Naegleriopel". This device is non-invasive and requires insertion into the nostrils at times of swimming or water sports related activities.

Methods: For prophylaxis of PAM, we propose a device called **"Naegleriopel"** that would block the pathogen at its portal of entry to the nose at the nostrils. This detachable device can be designed to be comfortable, cosmetically acceptable and easy to wear. Made up of synthetic plastic or silicone, this device would be adapted to the contours of the interior of the nose. As a nasal plug, this device with a convex round contoured tip and a hollow cavity could be easily inserted into the nostril bilaterally. The free ends of this connector bridge would be made to reach the centre of the concavity of a pair of nasal clogs. Each nasal clog would be made-up of expandable semi-firm, but of a soft silicone. These mushroom shaped nasal clogs connected at their centre of concavity to the central bridge would make the device insertable into the nostrils bilaterally. The connector ends could be glued or screwed to the clogs to prevent detachments. This device could be manufactured in three different sizes to suit different sizes of nasal apertures and age groups, which would enable its use in both, children and adults. The outer surfaces of the nasal inserts would be kept smooth and glistening to avoid irritation and subsequent sneezing or discomfort.

Results: This method is robustly expected to prevent the chance entry and access of Naegleria fowleri to the upper parts of the nasal cavity and lower down the scare of underwater swimming by preventing the entrance of contaminated water into the nose. It would help prevent the coughing and sneezing secondary to the water entry into the nose in individuals who are amateur swimmers and occasional swimmers. In areas of high incidences its free availability is expected to remarkably lower down the cases occurring annually.

Conclusion: This proposal could be tested in more human volunteers and could be evaluated for its compliance. Given the fact that Naegleria Fowleri has a mortality rate close to 99%, this device could offer a real protection of from the PAM and reduce the anxiety associated in the midst of swimming with head under the water.

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P-2: A Proposed Cascade Of Vascular Events Leading To Granulomatous Amoebic Encephalitis

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Objective: Granulomatous amoebic encephalitis due to *Acanthamoeba* is a chronic disease that almost always results in death (1). Hematogenous spread is a pre-requisite followed by amoebae invasion of the blood-brain barrier to enter the central nervous system (2).
Given the systemic nature of this infection, a significant latent period of several months before the appearance of clinical manifestations is puzzling (3). Based on reported cases, here we propose pathogenetic mechanisms that explain the above described latency of the disease.
Methods: Review of literature with details of the pathogenesis was explored and a model of infection was constructed. The sequence of event of *Acanthamoeba* in cerebral circulation was constructed.
Results: It was inferred that *Acanthamoeba* uses adhesion molecules and integrins to attach and invade the blood brain barrier and infect the brain. Also, there appears to be chemical chemotaxis the directs this protist towards the brain.
Conclusion: Knowledge of the pathogenetic steps involved in GAE could help manufacture drugs that could resist the CNS invasion after infection of soft tissue and skin by *Acanthamoeba* spp.

Keywords: GAE, Acanthamoeba, Blood Brain Barrier

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1. Baig AM, Iqbal J, Khan NA. In vitro efficacies of clinically available drugs against the growth and viability of an Acanthamoeba castellanii keratitis isolate belonging to the T4 genotype. Antimicrob Agents Chemother 2013;57:3561-3567.
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P-3: Evidence of a M1-Muscarinic GPCR homolog in unicellular eukaryotes: featuring *Acanthamoeba* spp. Bioinformatics 3D-modelling and experimentations

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Background: Acetylcholine affects the target cellular function via muscarinic and nicotinic cholinergic receptors that are seen to exist in humans. Both the cholinergic receptors are G-Protein coupled receptors (GPCRs) that perform cardinal functions in humans. Anti-muscarinic drugs, particularly the ones that target M1 subtype (mAChR1), have consistently shown to kill unicellular pathogenic eukaryotes like *Acanthamoeba* spp (1).

Objective: As the M1 receptor subtype has not been reported to be expressed in the above protist, the presence of an ancient form of the M1muscarinic receptor was inferred (2). Bioinformatic tools and experimental assays were performed to find the expression of ligand binding site.

Methods: A search for sequence homology of amino acids of human M1 receptor failed to uncover an equivalent ligand-binding site on *Acanthamoeba*, but structural bioinformatics showed a hypothetical protein L8HIA6 to be a structural homolog of the human mAChR1.

Results: Immunostaining with an anti-mAChR1 antibody showed cellular staining. Growth assays showed proliferation and lethal effects of exposure to mAChR1 agonist and antagonist respectively. With the recent authentication of human mAChR1 structure and its addition to the database, it was possible to discover its structural analog in *Acanthamoeba*; which explains the effects of anticholinergics observed in the past on *Acanthamoeba* spp.

Conclusion: With a narrow choice of drugs to treat fatal meningoencephalitis caused by *Acanthamoeba* spp, the discovery of an ancestral mAChR1 on *Acanthamoeba* could prove to be a potential therapeutic target in the future.

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2. **Baig AM, Iqbal J, Khan NA.** In vitro efficacies of clinically available drugs against the growth and viability of an Acanthamoeba castellanii keratitis isolate belonging to the T4 genotype. Antimicrob Agents Chemother 2013;57:3561-3567.

P-4: Evidence Targets of Aminodarone in *Acanthamoeba* spp. Bioinformatics and experimentations

***Dr. Abdul Mannan Baig*[1] , Anjia Tariq, Daniyal Mansoor, Amna Saeed, Mahrukh Usmani, Zohaib Rana and HR Ahmad**

[1]Aga Khan University, Stadium Road, Karachi, Pakistan

Background: Ion channels coupled to cell membrane receptors are seen in many multicellular eukaryotes to execute several metabolic and survival functions. Their role in the biology of the unicellular pathogenic eukaryotes, like *Naegleria fowleri* and *Acanthamoeba* specie has emerged recently (1).

Objective: Amiodarone, that binds potassium and calcium ion channels is being reported to be amoebicidal in pathogenic eukaryotes (2); therefore we hypothesized the presence of a homologous targets in *Acanthamoeba* spp.

Methods: Methodologies used were, bioinformatics analysis, structure activity relationship, ligand binding prediction and growth assays.

Results: We show the evidence for the presence of homology in targets of Amiodarone in humans with similar targets in *Acanthamoeba*. Growth assays with Amiodarone showed growth inhibitory effects.

Conclusion: Exploration of the targets of Amiodarone is expected to not only design better drugs for the treatment of diseases caused by *Acanthamoeba* but also would improve our understanding of the evolution of ion channels coupled to receptors in *Acanthamoeba* spp.

Keywords: *Acanthamoeba*, Amiodarone, Protein homology

1. Baig AM, Ahmad HR. Evidence of a M1-Muscarinic GPCR homolog in unicellular eukaryotes: featuring Acanthamoeba spp bioinformatics 3D-modelling and experimentations. *J Recept Signal Transduct Res*. 2016 Sep 7:1-9.

2. Baig AM, Iqbal J, Khan NA. In vitro efficacies of clinically available drugs against the growth and viability of an Acanthamoeba castellanii keratitis isolate belonging to the T4 genotype. Antimicrob Agents Chemother 2013;57:3561-3567.

P-5: *Acanthamoeba castellanii* secreted proteins enhance IL-10 production through the protein kinase A-dependent pathway in human monocytes

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Objective: Free-living amoeba of the genus *Acanthamoeba* can cause severe and chronic infections in humans which the most famous is *Acanthamoeba* keratitis (AK). The *Acanthamoeba* patients were also had reinfection and recurrences, suggesting that immunological memory is not developed in response to *Acanthamoeba* keratitis (AK). Previously study has found elevated IL-10 production in immune cells infected by *Acanthamoeba* that probably limits the inflammatory response. In this study, we focus on the signaling pathway stimulated by *Acanthamoeba* extracellular secreted proteins leading to IL-10 production in monocytes.

Methods: We collected the *Acanthamoeba* secreted proteins from *Acanthamoeba* cell-free conditioned medium (aCM) for stimulating THP-1 cell, a human monocytic cell line. The co-cultured cells were extracted the RNA and protein samples for analyzing IL-10, CREB, pCREB, PKA and pPKA expression by RT-PCR and western blot.

Results: The transcriptional activity of the IL-10 gene in the THP-1 cells is significantly augmented by stimulated with aCM. The expression level of the pPKA and pCREB are increased respectively in the stimulated THP-1 cells 4 and 8 hours.

Conclusions: Our results show that *Acanthamoeba castellanii* secreted proteins enhance the IL-10 production through the PKA-dependent pathway in THP-1 cells.

Significance: This finding presumed that the molecular mechanism of immune evasion of Acanthamoeba.

P-6: Oral contraceptives modulate the anti-inflammatory activity of *Acanthamoeba*-stimulated human macrophage

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PURPOSE. Macrophages are effector cells of the non-specific immune response involved in the first recognition and response to pathogens. Sex hormones may play an important role in regulating the immune responses and macrophages possess functional sex hormone receptors. Therefore, the aim of this study was to evaluate whether the use of oral contraceptives (OCs) can affect macrophage activity against *Acanthamoeba*. To this end, we evaluate the effect of OCs on pro-inflammatory and anti-inflammatory cytokines release by human macrophages in response to *Acanthamoeba*-derived cell-free conditioned medium (aCM) and to known agonists of TLR4 and TLR2 receptors (used as positive controls).

METHODS. Primary human peripheral blood monocytes from healthy adult women treated (FOCs) or non-treated (Fs) with OCs were isolated during the follicular phase of their menstrual cycle. Human monocyte-derived macrophages (MDMs) were obtained from each sub-population, and stimulated with either aCM, LPS or Pam2CSK4, *in vitro*. Release of TNF-α, IL-6, IL-10 and IL-8 was investigated at specific hours post-stimulation, by Enzyme Linked Immuno Sorbent Assay (ELISA). All experiments were performed in triplicate. Statistical difference was evaluated with the two-tailed Student *t* test and two-way analysis of variance (ANOVA), followed by Bonferroni post test, using Graph-Pad Prism (GraphPad Software Inc.).

RESULTS. Our data showed that by F-MDMs aCM induced a significant release of IL-6 and IL-10 throughout the time course; and IL-8 production at 18h post-stimulation. Of interest, a highly significant reduction of IL-8 and IL-10 was detected in FOC-MDMs in comparison with F-MDMs.

DISCUSSION. At the moment it is difficult to explain the impact that the reduced production of IL-8 and IL-10 observed in FOC-MDMs aCM-stimulated might have in *Acanthamoeba* infections. We hypothesize that the reduction of a already poor production of IL-8 might reduce macrophage ability to recruit neutrophils and other granulocytes in the site of infection, to the benefit of *Acanthamoeba*. On the other hand the reduction of IL-10 might promote the inflammatory response and the macrophage antimicrobial activity, to the detriment of the protozoan.

CONCLUSIONS. The use of OCs might influence the immune-modulatory activity of macrophages during *Acanthamoeba* infections.

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P-7: Detection Of *Naegleria* Species In Geothermal Springs In Italy

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Objectives: the purpose of this study was to conduct a local survey to identify at molecular level *Naegleria* species from two geothermal sites in Italy, for a better understanding of their ecology and to identify any potential health risks.

Methods: Water samples were taken between April, 2015 and July, 2016 from two geothermal spring recreation areas in Latium Region. Eight sampling points were selected. Two-liter of water were collected for each point and processed within 48h. Water temperature and pH were recorded *in situ*. Centrifugation of water samples was performed and cultures were done on Non Nutrient Agar-media containing *Escherichia coli*. All agar plates were incubated at 37°C and 45°C. Molecular characterization was obtained through DNA extraction and amplification using two sets of primers pairs, *Naegleria* genus-specific and *Naegleria fowleri* species-specific. Species identification was obtained by comparing the sequences with those available in GenBankTM through a phylogenetic analysis performed by MEGA6.

Results: Overall a total of 38 water samples (site A=25; site B=13) were collected and analyzed. Twenty-two out of 38 (57.89%) samples resulted positive for growth of *Naegleria* spp. At 37°C, the prevalence was highest in the site A (68%) in respect to the site B (38.4%). At 45°C, the percentage of *Naegleria* in site A was 24%, while no *Naegleria* grown was observed in site B.

Phylogenetic analysis allowed to identify in both sites: *Naegleria australiensis*, *Naegleria italica*, *Naegleria lovaniensis* and *Naegleria* sp.. The pathogenic *N. fowleri* was not detected. The most recurrent species was *N. australiensis.*

Conclusions and Significance of the work: The present study is the first molecular report on *Naegleria* spp. occurrence in geothermal hot springs in Italy. Interestingly, among the species identified, *N. australiensis* and *N. italica* were reported to be pathogenic in experimental animals. The relatively detection of potentially and not pathogenic *Naegleria* spp. in the water bodies studied is significant to public health, since these species are considered indicators of the potential presence of *N. fowleri* in such habitats.

P-8: Interactions of *Salmonella Typhimurium* mutants with amphizoic amoeba *Acanthamoeba castellanii*

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Free-living amoebas *Acanthamoeba* spp. are known as environmental reservoir for the intracellular pathogens, such as *L. pneumophila, M. avium, S.* Typhimurium. Survival of the pathogenic bacteria within protozoan hosts is a successful strategy of microorganisms to persist adverse environmental conditions. On the other hand, protozoan phagosomes also provide unfavorable conditions for ingested bacteria due to acid pH, iron and nutrients deficiency, impact of reactive oxygen.

In this work we aimed to study the ability of wild and mutant strains of *S.* Typhimurium 14028s to survive successfully within *A. castellanii* phagosomes. During the series of triple experiments the dynamics of CFU per ml *Salmonella* Typhimurium 14028S of wild type and four mutant strains has been registered. Every mutant strain contained deletion in a gene encoding stress response, such as ΔluxS :: KanR, ΔrpoS :: KanR, ΔrelA :: KanR and ΔyaiC :: KanR with failed induction of AI -2, synthesis of RNA polymerase sigma factor, ppGpp-synthase I, AdrA protein, respectively.

Axenic culture *A. castellanii* (ATCC® 30010™) was grown in the peptone–yeast extract–glucose (PYG) medium at 25 °C. S. Typhimurium 14028s (ATCC® 14028™) was grown in LB medium at 37 °C to late logarithmic phase, then washed with Page's Amoeba Saline (PAS) and added to the medium with *A. castellanii* washed with PAS, in a ratio of 10:1 bacteria per amoeba. *Acanthamoeba–Salmonella* co-cultures were grown in 1/10 medium PYG diluted with PAS at 30 °C. *Salmonella* viability was estimated by phase-contrast and fluorescence microscopy with vital staining, number of CFU was determined by plating on beef-peptone agar.

The results suggest that genes, which promote the survival of *Salmonella* under stress, are also involved in maintaining viability of the pathogen inside *A. castellanii* phagosomes.

Research was conducted in the Center of Shared Scientific Equipment "Persistence of microorganisms" of ICIS UrB RAS.

P-9: *Acanthamoeba* (T4) increases paracellular permeability and transepitelial resistance by modifying tight juntions composition without change MDCK epitelial cells morphology

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Free-living amoebae of the genus *Acanthamoeba* (T4) are cosmopolitan organisms, which can be isolated from diverse terrestrial and aquatic environments. Some species of the genus are potentially pathogenic for humans, provoking keratitis in healthy individuals, often among contact lens wearers, and opportunistic infections such as pneumonitis, fatal granulomatous encephalitis and skin infections, particularly in immunocompromised individuals.

At present the pathogenic mechanisms by which *Acanthamoeba* (T4) provoke cytophatic effect on target cells are not fully understand. Through *in vitro* assays it has been possible to suggest that contact-dependent factors play an important role during the invasion of these amoebae to target tissue, since the amoebae disrupt cells monolayers as well as corneal tissue and penetrate into deeper layers through cell-cell junctions, both by mechanical action of the trophozoites and the activity of proteases.

The aim of this work was to determine if these amoebae were able to damage the barrier function of the cell-cell adhesion tight junctions (TJ) in MDCK epithelial monolayers. Actin cytoskeleton integrity was evaluated by phalloidin-rhodamine staining and by electron microscopy; paracellular permeability and TJ sealing were studied by apicobasolateral diffusion of ruthenium red and transepithelial resistance (TER) measurements. Immunofluorescence and Western blot assays were performed to locate and estimate expression of TJ proteins claudins 2 (Cldn2) and 4 (Cldn4). Although were able to cross the MDCK monolayer, the amoebas did not alter the actin cytoskeleton or the morphology of the cells. Paracellular permeability was increased when amoebas or conditioned medium were present. After 6 hours of interaction, amoebas but not their conditioned medium induced an increase in TER, Cldn2 was removed from the TJ and its overall content in the cells diminishes, while Cldn4 was targeted to the TJ without changing its expression level.

In conclusion: *Acanthamoeba* (T4) crosses MDCK monolayer without damage the cells, increases permeability and TER through Cldn2 degradation and relocalization of Cldn4 to TJ. These results strongly suggest that contact-dependent mechanisms are relevant during amoebae invasion.

P-10: Contact lens-related infectious keratitis: review of 29 cases from Tunisia

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Purpose: To describe clinical findings, causative organisms and visual outcome of microbial keratitis associated with contact lens wear.

Setting: Department of ophthalmology, Fattouma Bourguiba University of Monastir, Tunisia between January 2006 and December 2016.

Methods: Retrospective review of the charts of 29 patients (29 eyes) with infectious keratitis associated with contact lens wear. All patients underwent detailed ophthalmic examination. Microscopic examination and culture of corneal scrapes, were performed in all patients. The contact lens and contact lens case were also examined, when available. Mean follow-up was 2.4 months (range: 1-12 months).

Results: Mean age of our patients was 27 years. Twenty-three patients (97.3%) were female and 6 patients (20.7%) were male. Twenty seven (93.1%) patients were soft contact lens wearers. Mean initial visual acuity (VA) was 20/800. Stromal infiltrates were associated with ulcers in all cases, and were located in central cornea in 21 cases (72.4%). Hypopion was noted in 10 cases (34.5%). Microbial cultures were positive in 18 patients (72.4%). Pseudomonas aeruginosa was isolated in 41.4% of cases. *Amoebic cysts were found in 5 cases (17.3%). Fusarium was isolated in one eye (3.5%).* Antimicrobial treatment was based on the suspected or isolated causative agent. Final VA was 20/125.

Conclusions: Microbial keratitis associated with contact lens wear, is a serious and increasing complication. Pseudomonas aeruginosa is the most common causative germ. Visual outcome is poor. Prevention and prompt management are mandatory to improve visual prognosis.

P-11: *Acanthamoeba* spp. Detection And Molecular Characterization In Stray Cats From Madrid, Spain.

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Introduction: Free-living amoebae including *Acanthamoeba* spp., *Naegleria fowleri* and *Balamuthia mandrillaris* are ubiquitous protozoan that have been isolated from air, dust, soil and water. They are considered opportunistic pathogens that affect both humans and animals, causing serious lesions in the eyes and the central nervous system. The prevalence of these emerging protozoan in domestic animals is unknown, so screening studies are necessary.

The aim of the present study was to determine the presence of *Acanthamoeba* spp., *N. fowleri* and *B. mandrillaris* in immunocompromised stray cats, considered potentially more susceptible to harbor the infection due to immune status and free access to contaminated environments.

Material and methods: A total of 60 cats included in a feral animal control program were analyzed. These cats were positive to retrovirus (feline immunodeficiency virus and/or feline leukemia virus). Corneal scraping and cerebrospinal fluid samples were obtained and subjected to plate culture for the isolation of the parasite. Molecular techniques (conventional and real time PCR) were done for detection of *Acanthamoeba* spp. *B. mandrillaris* and *N. fowleri*, followed by molecular characterization by PCR and sequencing.

Results: Two isolates (3.3%) of corneal scrapings were positive in culture, showing cysts and trophozoites of *Acanthamoeba* spp. These results were confirming by PCR and conventional real-time. One isolated was characterized as genotype T4 and the remaining one as T2.

Conclusion: This study reports for the first time the detection of *Acanthamoeba* spp. in immunocompromised stray cats in Spain. The presence of genotypes T4 and T2 of *Acanthamoeba* spp. suggest the significance for public health and veterinary medicine.

P-12: Comparison of Acanthamoeba PCR assays on human corneal samples for the diagnosis of Acanthamoeba keratitis

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Objective: The diagnosis of Acanthamoeba keratitis (AK) has been improved by the development of Nucleic Acid Amplification Tests (NAAT). However, the amplified sequences and conditions are different from test to test. In this study, we compared the performances of five published PCR assays on ocular samples for the diagnosis of AK.
Methods: The performances of standard PCR targeting different regions of the Acanthamoeba Rns gene with the Nelson, ACARNA, JDP1-JDP2 primers and real-time PCR assays targeting another region of the Acanthamoeba Rns have been prospectively compared on DNA extracted from 846 corneal samples from patients who were suspected to have AK.1,2 Ten patients had a definitive diagnosis of AK.1
Results: The sensitivities of standard PCR assays were 70%, 80% and 100% using JDP1-JDP2, ACARNA and the Nelson primers, respectively. Their specificities were 100%. The sensitivities of real-time PCR assays were 80% and 90% when the hybridization temperatures were 60°C and 63°C, respectively. Their specificities were 91% and 100% when the hybridization temperatures were 60°C and 63°C, respectively.
Conclusions: The diagnostic capacities of Acanthamoeba PCR assays depended on the targeted sequence and the amplification conditions. It could also probably depend on the diagnostic context (AK, granulomatous amoebic encephalitis or disseminated infection) as different genotypes of Acanthamoeba have been responsible for.
Significance of the work: As an early diagnosis and treatment warrants a successful outcome, a false negative result may have a negative impact on the prognosis of Acanthamoeba infections. Therefore specific and sensitive PCR are required for their diagnosis.

References:

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P-13: Detection of free-living amoebae isolates from regional hospitals in the Sierra Norte and Central Valleys of Oaxaca, Mexico

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Hospitals environments are places of special interest since hospitalized patients have a degree of immune compromise, which increases the risk of being infected by opportunistic Free-Living Amoebae (FLA).

The purpose of this study was to carry out sampling of FLA from 4 public hospitals in Sierra Norte and Central Valleys of Oaxaca, Mexico.

Samples were collected, using sterile swabs, from hospital environments, including: operating room, surgery table, water storage tanks, drinking fountains, nebulizers, tap water, air conditioners and patients shower water. FLA were isolated by culturing, using non-nutrient agar medium with *Enterobacter aerogenes*. Protozoans were identified in accordance with Page's morphological criteria (1988). Tolerance temperature tests were carried out for the isolates belonging to *Acanthamoeba*, *Naegleria* and *Vahlkampfia* genus.

36 samples (9 of each hospital) were collected from intrahospital environments, mentioned above. 12 samples were positive for FLA. Most of them were obtained from biofilms (7) and 5 from hydric samples. Among all the samples, 37% belonged to *Acanthamoeba* genus followed by *Naegleria* (19%) and *Vahlkampfia* (17%), *Echinamoeba, Gocevia, Hartmannella, Leptomyxa, Paratetramitus, Rosculus, Saccamoeba, Stachyamoeba, and Vannella* (27%).

We highlight the isolation of strains of *Acanthamoeba* given its clinical importance. Their prevalence in hospital environment may represent a risk health, particularly in immunocompromised patients as these protozoans are etiological agents of different pathologies; additionally they act as vector of diverse pathogens which aggravated the process.

Tolerance temperature test indicated that 4 of these *Acanthamoeba* isolates could be potentially pathogenic because they grew at high temperature (40 ^{0}C).

Biofilms are communities that allow the presence and permanence of diverse organisms potentially pathogenic for humans. The presence of FLA in hospital environments such as water and biofilms emphasizes the urgent need of implementing effective preventive measures. For our knowledge this is the first study which has been carried out in México. Further studies are required to estimate the true prevalence of FLA in Mexican hospitals by taking larger number of samples.

P-14: Epigenetic regulators for encystation of *Acanthamoeba castellanii*

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To transform into the resistant cyst stage of *Acanthamoeba*, the expression levels of a lot of genes mediating the encystation process have to be regulated during encystation. In higher eukaryotes, the study of epigenetic mechanisms responsible for various gene expression patterns has been widely used. Epigenetic regulation of gene expression is associated with DNA methylation, histone modification, and nucleosome positioning. Here, we reported the protein arginine methyltransferase 1 (PRMT1) and protein arginine methyltransferase 5 (PRMT5), known epigenetic regulators, in *A. castellanii*. And we investigated the correlation between the gene promoter methylation status and gene expression in *Acanthamoeba*. PRMT1 and PRMT5 were mainly localized in the nucleus, and highly expressed during encystation of *Acanthamoeba*. PRMT1 or PRMT5 knocked down *Acanthamoeba* by siRNA failed to form mature cysts. Promoter DNA methylation was measured by bisulfite sequence PCR, and the expression of cyst specific cysteine proteinase was regarded as promoter CpG island 1 hypomethylation. Detection of methylated cytosine in this study suggests the presence of a DNA methylation system for gene regulation in encysting *Acanthamoeba*. The findings of this study lead to a better understanding of the epigenetic mechanisms for the regulation of encystation in cyst-forming pathogenic protozoa.

P-15: Fatal granulomatous amoebic meningoencephalitis due to free living amoebae in two boys in two hospitals different in Lima- Peru

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Amoebic infections of the central nervous system (CNS) are very rare and usually fatal. Here we report two children with the history of headache, fever, sensorial hallucinations (olfactory and visual) and seizures, without cutaneous lesions in one of them, but the other, had an injury near the sacrum. The two boys were admitted with the diagnosis of acute meningoencephalitis and empiric therapy. The history of having been in contact with swimming pools and rivers, makes suspicion in an amebiasis. Although, trophozoites were never found in the wet mount of CSF. The clinical deterioration was evident. A brain computed tomography (BCT) and magnetic resonance image (MRI) with and without contrast, yielded diffuse cerebral edema with focal enhanced lesions and areas compatible with granulomatous lesion. Both developed brain injury evidenced in MRI. In both cases a brain biopsy was done, the histology confirmed granulomatous amoebic meningoencephalitis with the presence of amoebic trophozoites and occasional cysts. Unfortunately, the diagnosis was late and the both children died in the hospitals after almost two months of being hospitalized. The final diagnosis on the amoeba species of free living amoebae in these two patients will be done by real-time PCR and by 18S sequencing and fluorecence.

P-16: Free-living amoebae isolated from extreme environments

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Free living amoebae (FLA) are ecologically relevant protozoan, playing an important role regulating bacterial populations. Some species are of public health concern. It has been demonstrated that amoebae are able to tolerate adverse conditions, including high concentrations of disinfectants, a broad range of temperatures, UV light irradiation and can also grow and develop in extreme environments such as Arctic, Antarctica, caves and bat guano.

In this work the isolation of FLA from an artificial lake in Mexico, grown in extreme pH and high salinity conditions is documented.

Water samples were collected from an artificial lake located in the Ecological Park in Texcoco, Mexico, with the purpose to isolated *Arthrospira maximum* algae. Samples were cultivated in Zarrouk medium (high salt content) pH10, light trajectory of 7.5 cm, room temperature (25 to 30 °C), illumination 200 µmoles photons·$m^{-2}s^{-1}$, photoperiod 14/10 light/dark, and 200 ml·m^{-1}airflow. *A. maxima* mixed with diatoms, filamentous cyanobacteria and bacteria were observed. In order to inhibit the growth of these microorganisms, cultures were exposed to the following treatments:

Silica source was removed from Zarrouk medium causing the inhibition of the diatoms. Subsequently, cultures were exposed to thermal stress (55 °C/10 min), inhibiting filamentous cyanobacteria. Sodium chloride was then gradually added until reaching 800 mM, surprisingly enhanced the proliferation of flagellate and amoeboid protozoa, therefore, urea and ammonium chloride were added, resulting efficient only against flagellates. Finally, cultures were washed repeatedly at low speed with distilled water, however several weeks later high concentration of bacteria and amoebae proliferated again.

Amoebae were seeded in non-nutrient medium, incubated at room temperature and 30 °C, then were identified by morphological criteria according to Page (1988). *Pocheina rosea* y *Vermamoeba vermiformis*, grew at room temperature; *Acanthamoeba polyphaga* y *Vahlkampfia inornata* at 30 °C.

Acanthamoeba polyphaga is the etiological agent of corneal and CNS infections, *Vahlkampfia* and *Vermamoeba* have also been isolated from clinical samples even though their pathogenic potential is discussed.

The present study contributes with the knowledge FLA which are able to survive and grow in extreme culture conditions that allow them to have a wide distribution in nature, being a risk to health.

P-17: Free-living amoebae isolated from a lagoon Chinchaycocha in the highlands of the central Andes of Peru

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Free-living amoebae (FLA) are protozoa that are widely distributed in the environment mainly in water and soil related habitats. In this study, we have searched free- living amoebas in Andean lakes. The lakes in Peru are few, but we have one of the two largest in South America, Titicaca, with 8,380 km². The other, very important is Lake Junín or Chinchaycocha, located on the plateau of the same name, in the central highlands, about 4,140 meters above sea level. Lake Chinchaycocha, reaches an extension of about 32,000 hectares. Lake Chinchaycocha is in a eutrophic state, where large quantities of organic matter appear as a result of agricultural, urban and industrial discharges from the anthropic activities of the communities bordering the lake. We study the presence of free-living amoebas in this lake by two important facts; (I) there are large quantities of heavy metals, (ii) at 4100 meters above sea level. We take 50 ml of water from the pond and the samples were culture on non nutrient agar plates and checked daily for the presence of FLA, incubated at room temperature and 37 ° C. We found 5 strains of *Acanthamoeba* and 2 strain of *Leptomyxa*. Sp. This Acanthamoeba growth to 30°C and the *Leptomyxa* growth in 15 days to 30°C. Molecular characterization was carried out by amplifying the 18S rDNA gene and DNA sequencing, confirmed that the isolated strain belonged to Acanthamoeba. To the best of our knowledge, this is the first report of *Acanthamoeba* and *Leptomyxa* in Lake of South America- Peru over 4100 meters above sea level.

P-18: The DNA databases for the genus *Acanthamoeba*; an update to 2017

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The amoebae in the genus *Acanthamoeba* were discovered by Castellani and described by Volkonsky in 1930. Morphological characters (primarily cyst structure), and cytological characteristics of nuclear division (Pussard and Pons, 1977) were used to describe different species. Questions regarding morphological methods led to the use of molecular methods for determining more definitive subgeneric classifications. Initial 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 nuclear 18S rRNA gene led to the development by our lab of the sequence type classification now routinely used for classifying isolates of *Acanthameoba*. Since 1998, when 12 sequence types were identified, additional reports have elevated the number of types to as many as 22. In 2017, data on full or partial *Rns* sequences were available for over 3750 isolates of *Acanthamoeba*. Information from "almost complete" *Rns* sequences (sequences > 2000 bases) exist for over 400 isolates (a 40% increase since 2013), with 274 sequences identified from genotype T4 isolates (68%), a percentage similar to that seen for isolates with only partial *Rns* sequences. T4 is easily the most common sequence type seen in either environmental or clinical samples. The only other sequence type exceeding 5% of the total *Rns* sequences is type T5. Additionally, no new sequence type post-1998 represents more than 0.65% of isolates, except for type T15, associated with *A. jacobsi*. Many sequence types also contain significant phylogenetic subgroups (divergence between 1-5%). At least six significant subtypes exist within T4. Beyond a doubt, current species nomenclature within *Acanthamoeba* has little relationship to phylogenetic classification. Expansion of the databases included information on mitochondrial loci (over 130 mitochondrial *rns* sequences, 80 sequences for cytochrome oxidase subunit I, and complete sequences of the mitochondrial genome for 20 isolates). Multi-isolate information exists for a small number of nuclear proteins, including 50 sequence of beta-tubulin, 65 sequence of elongation factor-1, 55 sequences of glyceraldehyde-3-phosphate dehydrogenase, and over 30 sequences of 3 other genes. Information is summarized at our website: http://http://u.osu.edu/acanthamoeba/.

P-19: Transcriptomic analysis and profiling of differential expression genes between *Naegleria fowleri* trophozoite and cyst

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Naegleria fowleri, pathogenic free-living amoeba causing the fatal primary amoebic meningoencephalitis (PAM) in humans, is predominantly living in the lakes, rivers, swimming pools and contaminated drinking water. *N. fowleri* trophozoite can encyst to survive under the unfavorable conditions such as cold temperature, starvation and desiccation. However, the transcriptomic or genomic data in differential expression genes between trophozoite and cyst of *N. fowleri* is very limited. In this study, transcriptome RNA-sequencing libraries from *N. fowleri* trophozoite and cyst were investigated by de novo transcriptome assembly Next-Generation Sequencing (NGS) analysis. Databases, the assembly procedure resulted in mean full length of 11,254 nucleotides in total 42,220 transcript contigs and 37.21 % of C+G contents. As a result, RNA-sequencing transcriptome database indicated that upregulated 143 genes in cysts showed 2 folds expression in comparison with trophozoite and 163 genes were downregulated in comparison with trophozoite. These genes were found to participate in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. KEGG pathway included metabolisms(131), cellular processes(43), environmental information processing(22), genetic information processing(66) and organismal systems(20). On the other hands, by analysis of 10,713 sequences via the gene ontology database, their annotations included biological processes(1,069); cellular process(228), metabolic process(214) and single organisms process(193), molecular functions(415); catalytic activity(195) and binding(186), cellular components(923); possessing cells(240) and cell parts(225). In the increased differential expression transcriptome levels of *N. fowleri* cyst compared to trophozoite, protein kinase or lipid metabolisms related protein was upregulated. Especially, serine/threonine protein kinase or sphingosine plays a role in the regulation of cell proliferation, differentiation and death. Whereas cytotoskeleton related protein involved in the interaction of cell motility was upregulated in *N. fowleri* trophozoite. Finally, this study may provide new insights into the environmental resistant genes or pathogenic related genes in *N. fowleri* survival and infectivity.

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P-20: The identification of *Legionella* spp. and free-living amoebae (FLA) in water supply systems of hospitals by MALDI-TOF analysis and sequence-based typing (SBT)

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Legionella pneumophila is a bacterial pathogen of humans. Free-living amoebae (FLA) and ciliated protozoans can often serve as natural hosts and thus as reservoirs of these bacteria. Encysted amoeba can contribute to the resistance of intracellular *L. pneumophila* to various chemical and physical agents. Bacterial transmission to humans occurs through droplets generated from an environmental source such as air-conditioning units, shower heads, whirlpools, and other human-made devices that generate aerosols. The aim of this study was to investigate the presence of FLA and *Legionella* spp. in samples from water supply systems of hospitals using MALDI-TOF analysis and sequence-based typing (SBT). Our preliminary results suggest that both methods can be successfully applied for the identification and genotyping of FLA and *Legionella* spp. strains in samples from hospitals.

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P-21: Molecular isolation of free-living amoebae from Namhangang (South Han Liver) in South Korea

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Pathogenic free-living amoebae, *Naegleria fowleri* and *Acanthamoeba* spp. causing agents of an acute and lethal primary amoebic meningoencephalitis (PAM) and amoebic keratitis (AK) in humans, have been isolated from the natural environment. To ascertain the existence of free-living amoebae in Korean hydrosphere where water skiing and recreation have been actively performed, the collected water samples were subjected to molecular identification. The water samples of Yeoju city (around Namhangang) were collected from late August 2015 to August 2016, and then the non-nutrient agar culture and PCR-based detection technique were carried out. The collected surface waters were filtered with 0.45um pore-sized filter system, and then final samples were cultured on non-nutrient agar medium with inactivated *E. coli* and subjected to PCR with four kinds of primer pairs (P-FLA, NFA1, HART-NA and ITS primers amplify mainly the 18S-small ribosomal RNA or *nfa1* gene). PCR products obtained from water samples of Yeoju city were subjected to gene sequencing, and the similarity of 18S-rRNA sequences were compared with various reference amoebae in GeneBank data. In the results of genes similarity, the isolates showed 86-99% homology with *N. gruberi, N. philippinensis, N. australiensis, N. clarki, Acanthamoeba polyphaga, A. lugdunensis* and *Vermamoeba vermiformis.* Among them, two Korean isolates (confirmed by PCR as *A. polyphaga* and *A. lugdunensis*) have been axenically subcultured in PYG liquid medium at 30℃ incubator. In the in vitro cytotoxicity test, Korean isolate (tentative *A. polyphaga*) showed high cytotoxicity as much as reference amoebae, *A. polyphaga* and *A. castellanii*. Finally, various free-living amoebae were detected from Namhangang although pathogenic *N. fowleri* was not detected in the present study. These data will be useful for the detailed seasonal detection of free-living amoebae on a national scale in Korean hydrosphere in the further study.

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P-22: Isolation and biological characterization of *Acanthamoeba castellanii* from a case of keratitis in Mexico

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Amoebic keratitis (AK) is a sight threatening corneal infection with chronic course which frequently is misdiagnosed with herpetic or bacterial keratitis, moreover, if the diagnosis is carried out lately and the treatment is inadequately implemented, the infection can cause organ loss. Keratitis cases in Mexico are increasing, especially in young people contact lenses wearers.

In this work a 38-year-old woman with high myopia wearing contact lenses from Mexico City, referring severe pain and burning sensation in the left eye, with a history of wearing contact lenses while swimming is documented. Conjunctival hyperemia, and a central ulcer in cornea with irregular borders, infiltrates, edema, hypopyon and immune ring, with ciliary injection, was observed through the slit lamp. Laboratory samples were taken for viruses, bacteria, fungi and *Acanthamoeba*. Netilmicin 0.3%, oral itraconazole 100 mgs/12 h and tropicamide/phenylephrine was prescribed. The infection was resolved successfully after 8 months, leaving only a slight puncture outside the visual axis.

Clinical samples were positive only for amoebae: primary isolation was performed using 1.5% nonnutrient agar plates seeded with heat-killed *Enterobacter aerogenes* (NNA). Upon evidence of growth, cultures were established by transferring a single double walled cyst to fresh NNA plates. Amoeba was identified morphologically as *Acanthamoeba castellanii* according to the taxonomic criteria of the Page, and transferred to axenization in Bactocasitone medium (2%), determining an optimal temperature of growth at 30 °C. Amoebae virulence was evaluated by intranasal inoculation of 1 x 10^6/20 µl trophozoites in 2 groups of 5 of BALB/c mice, as well as by the interaction with monolayers of epithelial cells of the established MDCK line of canine kidney origin (1:2 ratio of interaction), at 1,3,6,8 and 24 h.

Acanthamoeba castellanii trophozoites were able to kill 50% of the inoculated mice recovering from brain, proving their invasiveness. Nevertheless, during the interaction with the MDCK cells, trophozoites migrated to cell junctions and scarce lytic zones were observed.

A. castellanii remains as one of the most frequently specie isolated from AK. In Mexico the diagnosis of AK cases is increasing. The treatment combining netilmicin and oral itraconazol contributed in the resolution of this pathology.

P-23: Identification of *Acanthamoeba* genotypes in the cornea samples of wild birds

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Objective: *Acanthamoeba* spp. is a free-living amoeba (FLA), which can be isolated from soil, waters, lakes, air, freshwater etc. is well known as an opportunist protozoan parasite causing infections in humans and animals. *Acanthamoeba* spp. has been isolated from variety of organisms including insects to humans. *Acanthamoeba* spp. can lead to infection in the eye, the central nervous system, lungs and skin. The aim of this study was to isolate *Acanthamoeba* spp. from cornea samples of wilds birds using agar plate, PCR and sequencing them for genotyping.

Methods: Wild birds (n: 18) used in this study were found dead in Aegean region of Turkey and brought to the İzmir Natural Life Park and Izmir Bird Paradise. The corneas of the birds were examined for the existance of keratitis. Then, the corneas of each bird were aseptically removed and a part of the cornea was transferred to 1.5% non-nutrient agar plate covered by heat-inactivated *Escherichia coli.* The plates were incubated at 30-32°C for 15 days. Each plate was examined daily under inverted microscope to check to the presence of *Acanthamoeba* spp. Remaining part of the corneas were used to isolate DNA by QIAamp DNA Mini Kit according to the manufacturer's protocol. Then, PCR targeting 18S rRNA gene (GenBank no: U07413) was used to amplify ~229 bp region using the Nelson 1 (5′-GTTTGAGGCAATAACAGGT -3′), Nelson 2 (5′- GAATTCCTCGTTGAAGAT -3′) primers and was used to amplify ~450-500 bp region using the JDP1 (5'-GGCCCAGATCGTTTACCGTGAA-3'), JDP2 (5'-TCTCACAAGCTGCTAGGGAGTCA-3') primers. *Acanthamoeba* spp. positive samples were sequenced and genotyped.

Results: Keratitis was detected in two Eurasian sparrowhawks (*Accipiter nisus*) based on external examination of the corneas. *Acanthamoeba* spp. was isolated from a Eurasian sparrowhawk with keratitis by non-nutrient agar plate method and PCR. PCR detected *Acanthamoeba* spp. DNA in another Eurasian sparrowhawk with keratitis and a Peregrine falcon (*Falco peregrinus*) without keratitis. Altogether, *Acantamoeba* spp. was detected in three wild birds (16.6 %). Among the positive samples, two of them were genotype T5 and the remaining was genotype T4 based on sequencing results.

Conclusion: Previously, *Acanthamoeba* spp. was detected in liver of birds. In the present study, *Acanthamoeba* spp. was detected for the first time in the eye of two Eurasian sparrowhawks and a Peregrine falcon. One of the source of keratitis in wild birds can be *Acanthamoeba* infection resulting with insufficient feeding and consuquently death of the bird. These reults also show that the cause of Acanthamoeba infection in humans can be related to the wild birds.

Keyword: *Acanthamoeba* spp., wild bird, PCR, agar plate

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P-24: Identification of free-living amoebae isolated from tap water in Istanbul, Turkey

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Objectives: Free-living amoebae (FLA) are widely spread in the environment and also known to cause rare but often serious infections. The present work focuses on a local survey on FLA. It is essential to know the prevalence and distribution of these microorganisms in order to get infections caused by them under control.

Methods: In this study, FLA isolated from domestic tap water samples from homes of contact lens wearers were identified by morphology and by 18S rRNA gene sequence analysis.

Results: Morphological analysis and partial sequencing of the 18S rDNA revealed the presence of *Acanthamoeba* genotype T4 and *Vermamoeba vermiformis* in the investigated tap water samples. *Naegleria fowleri*, *Balamuthia mandrillaris*, and *Sappinia* spp. were not detected during this study.

Conclusions: It was shown that species of FLA known to cause eye infections in humans are widely distributed in tap water in Istanbul, Turkey. Contact lens wearers should be aware of the risk of contamination from tap water and strictly apply stringent contact lens hygiene. With this study we established *Acanthamoeba* genotype T4 and *Vermamoeba vermiformis* as contaminants of tap water in Istanbul.

P-25: Prevalence of Free living amoebae in the domestic water reservoirs in Sfax, Tunisia

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Free living amoebas (FLA) are opportunistic pathogen found in different water sources in the environment. The aim of this study was to investigate the prevalence of free living amoeba in different samples of domestic water reservoirs (DWR) in Sfax(Tunisia). It was a prospective study dealing with 486 water samples collected from different DWR. After filtration through a cellulose acetate membrane, samples were cultured on non-nutrient agar and the FLA were detected and strained by Giesma, Trichrom and red nuclear stain for morphological and morphotypic studies. FLA was found in 62% of samples with one morphotype or more. The acantopodialmorphotype was detected in 43%, polytactic in 38%, monotacticin 28%, fan-shaped in 17%, rugose in 11%, dactilopodial in 10% and eruptive in 9% of samples. These results demonstrate that domestic water reservoirs are a significant source of microorganisms and well maintenance of DWR is recommended.

P-26: Molecular characterization of free amoebae responsible for Keratitis diagnosed at the La Rabta hospital in Tunis

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Amoebic keratitis (AK) is a rare, but a serious and painful sight-threatening disease that can lead to very heavy visual sequelae.Early diagnosis and appropriate treatment are imperative.
The aim of this work was to identify the genotypes of *Acanthamoeba* isolated from patients with amoebic keratitis diagnosed in the laboratory of Parasitology at the Rabta Hospital of Tunis.
This was a retrospective study over a period of 7 years (2009-2015) including 25 positive agar cultures showing amoeba cysts. These specimens involved 14 patients followed for infectious keratitis: 14 lenses, 10 case waters and a pus sampling of a corneal abscess were analyzed by direct examination, culture on Sabouraud medium and PCR. Ten positive PCR could be sequenced. The T4 genotype was isolated in 9 cases (9 samples from 5 patients) and another patient carried the T6 genotype.
In conclusion, the T4 genotype is the cause of amoebic keratitis most frequently isolated in Tunisia. This amoeba occurs naturally in the environment and represents a potential reservoir and source of infections in humans, Nevertheless, further studies should be developed in order to establish the pathogenic potential of the isolated strains.

P-27: Anti-*Acantamoeba* activity of citrus peels essential oil and the effect of viroids infection

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In order to valorize a local Tunisian product, this study was designed to examine for the first time the anti-*Acantamoeba* activity of Tunisian citrus *Sinensis* peels essential oil (Maltaese half-blood) and the effect of viroids plant infection on this activity. For this purpose three samples of peel's essential oils were studied: the healthy plant noted (T), plant inoculated with exocortis (CEVd) noted (1) and plant inoculated with cachexia (HSVd) noted (4).
The samples were extracted by hydrodistillation from dried peels and characterized by GC–MS. Limonene was the major component with a percentage ranged from (90.76 – 93.34%) for SO 1 to SO T respectively.
Anti- *Acanthamoeba* activity of the assayed oils was determined by Alamar Blue assay. Primary results showed a strong anti-*Acantamoeba* activity potential with an IC_{50} ranged from (36.6 – 54.58 µg/ml) for SO 4 to SO 1 respectively.
Regarding the effect of viroid infection, a strong positive correlation between the amount of oxygenated compounds and anti-Acantamoeba activity was observed.

Keywords: *Acanthamoeba*, Maltease, Essential oil, viroids

P-28: *Melalleca styphelioides* essential oil: Chemical composition and Anti-*Acantamoeba* activity

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Melaleuca styphelioides (Myrtaceae family), a plant native to eastern Australia, It is also one of the most important essential oil-producing species. Previous studies reported a broad spectrum of biological activity such as anti-bacterial and anti-fungical activities [1,2].

The objective of the present study was to evaluate the chemical composition of the essential oil obtained from the leaves of *M. styphelioides* by hydrodistillation and to investigate their anti-Acanthamoeba activity. Identification and quantification were realized by gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection by (GC-FID). The major compounds identified were: Caryophyllene oxide (23.42%), Spathulenol (20.5%), Isoaromadendrene epoxide (7.45%) and Ledol (5.98%). The essential oil was characterized by a high percentage of sesquiterpenes (63.29%).

The Alamar Blue Cell Viability Assay was used to evaluate the "*In vitro*" anti-*Acanthamoeba* activity of *M. Styphelioides* essential oil. The results showed that the essential oil found to have a moderate activity with an IC_{50} = 69,03 ± 9,17 µg/mL.

Keys words: *Melaleuca styphelioides*, essential oil, GC-MS, Acanthamoeba castellani.

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P-29: Activity of *Ammoides pusilla* essential oil and extracts against *Acanthamoeba castellanii* neff

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Ammoides pusilla is an annual plant belongs to the family *Apiaceae*. It is locally known as "Nûnkha". This plant had aromatic and medicinal properties. It had been used to treat several diseases such as headaches, fever and diarrhea [1].

The main objective of the present study was to evaluate the chemical composition of essential oil obtained from the leaves, flowers and aerial part of *A. pusilla* by an alternative method "Hydrodistillation". Identification and quantification were realized by gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection by (GC-FID). GC/MS analysis of essential oils showed that the main components were Thymol (41.27% and 33.05 %), ϒ-terpinene (28.57% and 28.19%) and p-cymene (12.21% and 15.31%) for leaves, flowers and aerial part, respectively. The antiparasitic activity of essential oils was evaluated against *Acanthamoeba castellanii neff* by the Alamar Blue assay. Results showed that essential oil of leaves and flowers of *A. pusilla* essential oil (IC_{50}= 65.32 ± 5.43 µg/mL) was more active than aerial part (IC_{50} = 97.18 ± 1.43µg/mL.

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P-30: Studies on essential oil composition and anti Acanthamoeba Activity of *Teucrium ramosissimum*

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The medicinal plants contain essential oils and other substances that can be used in foods, perfumery aromatherapy, herbal medicines or cosmetics **[1]**. *Teucrium ramosissimum* (family-*Lamiaceae*), is a perennial herb endemic in North Africa and commonly known as "*hchichet belgacem ben salem*" **[2]**. The aim of the present study was to evaluate the chemical composition of the essential oil obtained from the aerial parts of *T. ramosissimum* by hydrodistillation and to investigate their anti-Acanthamoeba activity. Identification and quantification were realized by gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection by (GC-FID).
97.78% of the essential oil representing 68 compounds was identified. The major compounds were: δ-cadinene (18.63%), δ-cadinol (18.70%), β-eudesmol (12.13%), γ-gurjunene (4.34%) and 8-cedrene (3.99%). The essential oil was characterized by a high percentage of sesquiterpenes (80.62%). Oxygenated sesquiterpenes were present at 46.69%. The hydrocarbon sesquiterpenes were less abundant (33.93%). The monoterpene fraction of essential oils was less than 14.34%. Many compounds were identified for the first time in this essential oil where eight are the most abundant (% > 2). The findings of the anti-Acanthamoeba assay indicate that *T. ramosissimum* essential oil found to have a highest activity with an $IC_{50} = 25.73 \pm 0.75$.

Keys words: *Teucrium ramosissimum*, essential oil, GC-FID/GC-MS, Acanthamoeba castellani, .

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P-31: Anti-*Acanthamoeba* activity of *Thymus capitatus* essential oil and extracts

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Abstract:

Acanthamoeba species are free-living amoebae widely present in the environment which threatens human health. The treatment of infections *caused by Acanthamoeba* is always complicated and not obviously effective. To fight *Acanthamoeba*, medicinal plants could be considered as a potential source for more efficient treatment. Our research focused on the investigation of the anti-*Acanthameoba* activity of the essential oil and the ethanolic extract obtained from *Thymus capitatus L.* Pure compounds were isolated from this plant by column chromatography and were analyzed by NMR spectroscopy. Essential oil showed best activity with an IC_{50} of 2.73 µg/ml. The conducted Bio-guided fractionation of the thyme extract result in the identification of two active compounds namely thymol and 2,3-dihydroxy-*p*-cymene. The results have clearly shown that the investigated products may be efficiently used against *Acanthamoeba* infections. These molecules that are found in the plant may constitute a natural alternative for the development of new drugs.

Keywords: *Acanthamoeba*; *Thymus capitatus*, Essential oil, Thymol, 2,3-dihydroxy-*p*-cymene.

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