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DR. JULIO MARTINEZ (1930-2002) AND HIS CONTRIBUTION TO THE PATHOGENIC FREE-LIVING AMEBA RESEARCH WITH SPECIAL REFERENCE TO AMEBIC ENCEPHALITIS CAUSED BY *Balamuthia mandrillaris.*

Govinda S. Visvesvara[1] *and Frederick L. Schuster*[2]

ABSTRACT

Dr. Augusto Julio Martinez passed away at the age of 72 years on December 27, 2002 just two days after Christmas, the time of the year he liked so much. Dr. Martinez was professor emeritus of neuropathology at the University of Pittsburgh Medical School, Pittsburgh, PA where he was consulted by physicians and researchers from all over the world, to help diagnose brain disorders such as Alzheimer's, CJK and of course amebic meningitis. Julio was born in Cuba and obtained his early education including the medical degree in Cuba. After Fidel Castro and his communist forces overthrew the government and replaced it with the oppressive totalitarian regime Julio left Cuba as a stowaway on a ship to Spain. He came to the United States from Spain and completed his postgraduate training at the Case Western Reserve University in Cleveland, OH. He met and married Josephine, daughter of Irish immigrants and started to raise their family. Eventually in 1972, he moved to Medical College of Virginia via the University of Tennessee and began his love/hate relationship with the small pathogenic free-living amebas. Although he had no training in protozoology he loved the amebas because of their ability to live in all kinds of environments and their incredible virulence properties, but hated them because of the anxiety and despair they caused to the families of young children who succumbed to these amebas. He became obsessed with these amebas and developed a research program in Virginia to study their pathogenic properties. His first encounter with these amebas came in ~1969 when he learned of the deaths of several children who became sick and died within a few days of swimming and playing in a neighborhood lake. He visited, with his family, the lake in which the children swam and examined the environment and surroundings where families with young children enjoyed picnics. He has very meticulously described these sites in his comprehensive book on these free-living amebas (Martinez, 1985).

He had the uncanny ability of distinguishing between cells that were very similar to amebas as well as recognizing amebic organisms in tissue sections at very low magnification. Therefore, he came to be known as a stellar diagnostician. Because of this expertise he was considered a benchmark, a yardstick, a legend not only in neuropathology but also in free-living ameba

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research. He published more than 200 papers of which more than 50 papers were devoted to ameba research. He was instrumental in advancing our knowledge on the pathogenesis of *Naegleria fowleri, Acanthamoeba* species and *Balamuthia mandrillaris*. He showed by mouse model experiments and electron microscopy that *N. fowleri,* after entry into the nasal passages, interacted with the sustentacular cells and thereafter made their way into the olfactory lobes via the cribriform plate. His seminal paper "Is *Acanthamoeba* encephalitis an opportunistic infection" (Martinez, 1980) paved the way for diagnosing more and more cases of *Acanthamoeba* encephalitis, and skin infections. He made an important distinction between infections caused by *N. fowleri* as primary amebic meningoencephalitis, and those caused by *Acanthamoeba* as granulomatous amebic encephalitis (GAE). Later on when *Balamuthia mandrillaris* was discovered he showed that *B. mandrillaris* like *Acanthamoeba* caused GAE. He was again the first to help develop an animal model using SCID mice to study pathological aspects of *B. mandrillaris* infections.

PRIMARY AMEBIC MENINGOENCEPHALITIS DUE TO *Naegleria fowleri*: THE FIRST ITALIAN CASE.

Massimo Scaglia[1], Simonetta Gatti[2], Paola E.Cogo[3], Rita Alaggio[4], Flavio Rossetti[5], Nicoletta Mainini[3], Franco Zacchello[3] and Govinda S.Visvesvara[6]

ABSTRACT

We report here the first case of primary amebic meningoencephalitis (PAM) in Italy. The etiologic agent was identified, most probably, as *Naegleria* based on the clinical history and the fresh water exposure as well as the presence of numerous amebic trophozoites measuring 8 to 10 μm and no cysts in the brain tissue sections. Indirect immunofluorescence test using polyclonal rabbit anti-*N. fowleri* serum identified the amebas as *N. fowleri*.

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IMMUNOHISTOCHEMICAL STUDY OF PRIMARY AMEBIC MENINGOENCEPHALITIS IN MICE.

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ABSTRACT

The migratory pathway of *Naegleria fowleri* from the nasal submucosa to the central nervous system (CNS) during the early stage of primary amebic meningoencephalitis (PAM) was investigated in mice. Six weeks old Balb/c mice were inoculated by intranasal instillation of 1 x 10^6 trophozoites. Groups of at least 3 animals were sacrificed after 8,12, 24, 30, 48, 72, 96 and 120 h post-inoculation. The heads were decalcified and embedded in paraffin and processed to obtain 7μ slices in a traverse plane. The slices were deparaffinated and moisturized until PBS, to blockade the endogenous peroxidase. The samples were incubated with bovine serum and later on the samples were incubated with polyclonal serum of rabbit anti-*N. fowleri* for 1 h, and they were incubated with the second antibody of goat anti-Ig of rabbit conjugated to peroxidase. The activity of the peroxidase was revealed with H_2O_2-DAB. The samples were counterstained with Harris' hematoxylin. We concluding the inmunoperoxidase is a reliable tool for the studies of the PAM produced by *N. fowleri*, and that the first barrier that the parasite has to surpass before invading the epithelium is the superficial mucus. It rest to be detrmined the factors that allow the parasite to make that first contact with the layer of mucus of the olfactory epithelium, and to establish the possible role of the mucus as a defense barrier; it would be also of interest to establish which factors allow the parasite to surpass the mucus and make contact with the epithelial cells.

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SUCCESSFUL TREATMENT OF *Naegleria fowleri* MENINGOENCEPHALITIS BY USING INTRAVENOUS AMPHOTERICIN B, FLUCONAZOLE AND RIFAMPICIN. CASE REPORT.

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INTRODUCTION

Primary amebic meningoencephalitis (PAM) caused by *Naegleria fowleri* is a generally rapidly fatal disease, which develops in individuals who in the past few days swam into water bodies containing this free living ameba (FLA) (Barnett *et al.*, 1996; Hannish and Hallagan, 1997; Parija and Jayakeerthee, 1999).

Even though PAM is still considered a rare disorder, the number of reports increases each year, with fatal outcome in almost all cases; we've compiled only 8 reports in which cure was achieved (Anderson *et al.*, 1973; Apley *et al.*, 1970; Brown, 1991; Jain *et al.*, 2002; Loschiavo *et al.*, 1993; Poungvarin and Jariya, 1991; Seidel *et al.*, 1982; Wang *et al.*, 1993), one of them under impugnation (Brown, 1991). In Mexico, there have been over 25 cases informed, most on the northwestern region of the country (Lares-Villa, 2001; Lares-Villa *et al.*, 2001; Lares-Villa *et al.*, 1993; Valenzuela, López-Corella and De Jonckheere, 1984), with only one successful treatment known (Rodríguez-Perez, 1984).

Amphotericin B remains the cornerstone of treatment, alone or in combination with other drugs (Jain *et al.*, 2002), but no treatment has been scientifically validated because of the low incidence and lethal course of the disease. We herein report a case of PAM which we've recently had the opportunity to treat within the very first hours of its evolution, in which we succeeded in promoting a complete recovery of our patient.

Case report

A 10 year old boy, previously healthy and with no history of disease among relatives, was admitted to the emergency room last April, because of persistent severe headache, vomiting and fever. One week before he had gone swimming in a nearby irrigation canal.

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In vitro INTERACTION OF *Acanthamoeba castellanii* WITH CORNEAS FROM DIFFERENT EXPERIMENTAL ANIMALS.

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ABSTRACT

Acanthamoeba keratitis (AK) is a corneal infection produced by the worldwide distributed free-living protozoan of the genus *Acanthamoeba*. The mechanisms and factors involved during the process of corneal invasion, especially those related with the early stages of amoeba-cornea interaction are poorly understood. In this work, we determined the possible affinity of various laboratory experimental animals to ocular *Acanthamoeba castellani* infection. We also analyzed some of the morphological events occurring during the early stages of host-parasite interaction.

One million trophozoites of *A. castellanii* strain were interacted during 4 h with intact corneas obtained from Balb/c and C3H mice, Wistar rats and rabbits. For control, similarly obtained corneas were interacted only with the culture medium. Samples from selected areas were fixed, embedded in epoxy resin and semi-thin sections were stained with toluidine blue and examined under a light microscope.

At 4 h of interaction, trophozoites of *A. castellanii* were able to adhere and produce superficial erosion of the corneal epithelium in all animals studied. Non specific changes were observed in epithelial cells localized at some distance form the parasite.

To determine the final destiny of the parasites in each animal species, and whether mechanical or enzymatic activity is participating during the adhesion and penetration of the amoeba to the target organs, we are currently performing biochemical, electrophysiological and ultrastructural studies at this short period and at longer interaction times.

Key words: *Acanthamoeba* keratitis, animal models, corneal damage, light microscopy.

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ISOLATION OF *Acanthamoeba* FROM NORTH SEA COASTAL ENVIRONMENTS.

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ABSTRACT

Acanthamoeba are free-living protozoa that are ubiquitous in nature. Some species within this genus are opportunistic pathogens of man. *Acanthamoeba* have been isolated form a variety of sources including fresh and marine waters. A range of 'pollutants' often affects these waters. These environments are often used for recreational purposes and may represent a potential source of infection. In this study we assess the impact of 'pollutants' on the isolation rates and species/pathogen distribution of *Acanthamoeba* in a number of North Sea coastal sites. Samples were taken at 6 sites along the North Sea coast; the Humber estuary, South Bay Scarborough, Whitby, Tynemouth, the Forth estuary and the Tay estuary. Acanthamoeba were isolated from these sediments using bacterial overlay plates. From initial isolates, *Acanthamoeba* where subcultured then identified/ speciated using cyst morphology and *Hae III* restriction of a ribosomal DNA PCR product. The pathogenicity of isolates was assayed by culture on high osmolarity plates and by growth at 37^{0}C. Environmental data for each site was obtained from the Environment Agency and the Scottish Environmental Protection Agency. *Acanthamoeba* were isolated from 34 of 41 sediment samples. The majority of isolates (65/77) were from groups II & III based on morphology and 42 were speciated using restriction analysis. From the data collected it would seem that environment has a significant effect on the diversity of species isolated with species diversity being reduced in polluted environments. Interestingly the majority of pathogens were isolated from 'clean' waters

Key Words: Cysts, pathogen, pollution, species.

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CHARACTERIZATION OF *Balamuthia* AMEBAS ISOLATED FROM THE ENVIRONMENT.

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ABSTRACT

The *Balamuthia* amebas are recognized as the causative agent for the usually fatal disease, granulomatous amebic meningoencephalitis. First isolated from the brain of a Mandrill baboon in the San Diego Zoo, the amebas now have been found in a variety of mammals. In humans, some 90 cases have been identified in persons, young and old distributed around the world. Recently, we reported the isolation of an ameba (RP5) from the home of a child who died of amebic encephalitis. Here we report the finding of another ameba (OK1) obtained from a different unrelated location and the comparison of the two environmental isolates with the clinical isolate from the child (SAm). The procedure for the recovery of the amebas from soil samples and the comparison of the three amebas include observations made on 1) their growth conditions, 2) their fine structure observed by electron microscopy, 3) their indirect immunofluorescent response to *Balamuthia* antibodies, 4) their susceptibility to antimicrobials, and 5) the sequence analysis of their DNA. The evidence is consistent that these two environmental isolates as well as the clinical isolate are *Balathumia* amebas.

Key words: amebic encephalitis, clinical isolate, fatal disease, soil samples.

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CHARACTERIZATION OF THE *Naegleria* ISOLATES FROM THERMAL WATERS IN JAPAN.

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ABSTRACT

In an attempt to isolate virulent species of the genus *Naegleria*, varieties of *Naegleria* strains were isolated from public bath water and thermally polluted sewage. Among 549 water samples from 251 sites, approximately 36% of them were positive for amoebae that grow at 42°C. By the morphological characterization, a total 1,093 clones of amoebae belonged to the genus *Naegleria* were isolated. PCR/RFLP analysis using ITS regions as the target molecule revealed the occurrence of *N. lovaniensis* (46%) or *N. australiensis* (37%) as the dominant species in the environmental thermal waters in Japan. The latter is known to be pathogenic, but only one isolate killed mice (5 / 5) consistently within 3 to 9 days when the amoebae were injected intracerebrally at a concentration of 10^4 cells per mouse. The amoebae recovered from brain samples of the dead mice were confirmed as *N. australiensis* by DNA sequences of the ITS regions.

Key words: IEF, ITS, *Naegleria australiensis*, pathogenicity, PCR/RFLP.

INTRODUCTION

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FREE-LIVING AMOEBAE FROM A DOMESTIC WASTEWATER TREATMENT SYSTEM OF A SINGLE-HOUSE.

Elizabeth Ramirez[1], Esperanza Robles[1], Patricia Bonilla[1], David Gomez[1] and Alan Warren[2].

ABSTRACT

Free-living amoebae occur in habitats affected by sewage. Sewage is an important source of potentially pathogenic FLA. The aim of the study was to determine the presence and numbers of free-living amoebae in a domestic wastewater system (RZM).

The RZM system selected for the study was fed with raw wastewater from a five-people single house without drainage. Inlet, outlet and middle of the bed samples were taken of January of 2001 to February of 2002 monthly. Most probable number method (MPN) was used for enumerating FLA, identification of the amoebae were made on the basis of morphological features.

Thirty four species of FLA representing 14 genera were isolated from the reed bed. *Acanthamoeba* was the most frequent (38.2%). The persistence of this genus in wastewater has been reported before, probably is related to the resistance of cysts to desiccation, freezing temperatures, and commercial disinfectants.

Maximum abundance reached 2 400 000 amoebae L^{-1} and minimum abundance was 2100 amoebae L^{-1}. The middle of the bed had the major diversity with 32 species and mean abundance of 598 145 amoebae L^{-1}, followed by the inlet with 24 species and 105 625 amoebae L^{-1}, and the outlet with 20 species and 46 466 amoebae L^{-1}. Maximum abundances occurred in the spring and minimum were found in winter, probably because it is an opened system and is affected by the environmental conditions, this agreed with the known seasonal patterns of the amoebae. Positive correlations between amoeba abundance and temperature, pH and conductivity were found.

Key words: enumeration FLA, MPN, reed bed, root zone method, sewage

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CURRENT PERSPECTIVES ON *Acanthamoeba* KERATITIS IN INDIA: PREDISPOSING FACTORS, MICROBIOLOGY, HISTOPATHOLOGY AND GENETIC CHARACTERIZATION.

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ABSTRACT

This study reports clinical, microbiological, histopathological and molecular features of *Acanthamoeba* keratitis seen at a tertiary eye care center in southern India. Beginning 1995, all patients diagnosed to have *Acanthamoeba* keratitis on the basis of positive smears or culture of corneal scrapings were included in the study. Medical records, microbiological data and histopathological data were retrospectively analyzed. Selected number of isolates, were used for molecular characterization.

Between January 1995 and May 2003, a total of 173 patients were diagnosed to have *Acanthamoeba* keratitis. Only one out of 173 (0.6%) patients had a history of contact lens wear while trauma to cornea and/or washing of eyes with contaminated water was the risk factor in majority of the other patients. Calcofluor white, Gram and Giemsa staining of corneal scrapings established the diagnosis in 89%, 81% and 73% of cases respectively. Monoxenic culture was positive in 81% cases. All patients were treated with 0.02% polyhexamethylene biguanide and /or chlorhexidine. Eighteen out 173 (10%) patients required penetrating keratoplasty/evisceration. Histopathology of corneal buttons revealed necrotising stromal inflammation in most cases. In five cases a rare observation of granulomatous inflammation with a immunophenotype of T cell, CD 68 marker positive and B cell negative was made. Thirteen amoebic isolates from non-contact lens associated keratitis along with several reference strains were analyzed for nucleotide variation in partial or complete 18S rRNA gene. Phylogentic analysis showed that all isolates were *Acanthamoeba* carrying T4 genotype signature sequences. Further comparison revealed that partial sequencing was sufficient to distinguish closely related strains of *Acanthamoeba*.

Key words: Genotyping, Granulomatous, Non-contact lens wearers.

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STUDIES ON THE SYSTEMATICS, PHYLOGENY AND PATHOGENICITY OF *Naegleria* USING RIBOSOMAL DNA SEQUENCES. A TRIBUTE TO THOMAS BYERS, 25 YEARS AFTER THE FIRST '*Acanthamoeba*' CONFERENCE IN COLUMBUS, OHIO.

Johan F. De Jonckheere [1*]

ABSTRACT

My laboratory has used ribosomal DNA (rDNA) sequences for the identification, phylogeny and systematic study of *Naegleria* over the past decade. While at first I used small subunit (SSU) rDNA, over the years, I have turned my attention to the internal transcribed spacer (ITS) and 5.8S rDNA sequences. Phylogenies based on ITS and 5.8S rDNA match those based on SSU rDNA. Sequencing ITS for the identification of *Naegleria* spp. has now been adopted by other laboratories across the world. At present 24 *Naegleria* spp. have been described using a variety of different methods, and 11 new species are proposed in a recently submitted manuscript. All 35 species can be identified on the basis of their ITS rDNA sequences. Using this technique I was able to determine the species identity of strains of which DNA or cell pellets (or even protein extracts containing small remains of DNA) I discovered in my freezer; these samples were up to 20 years old. Using this precise molecular definition of a species I have been able to study the global dispersal of *Naegleria* spp. This study has since been extended to include the other vahlkampfiids.

Key words: group I intron, internal transcribed spacers, 5.8S rDNA, small subunit, large subunit, new species, vahlkampfiids.

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MULTIPLE ALLELES OF *Acanthamoeba* NUCLEAR SMALL SUBUNIT RIBOSOMAL RNA GENE: FURTHER EVIDENCE OF POSSIBLE GENETIC EXCHANGE BETWEEN CLOSELY RELATED STRAINS.

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ABSTRACT

The nuclear small subunit ribosomal RNA (*Rns*) gene has been used in the last decade to establish genotypes of the genus *Acanthamoeba* based upon these sequences. In some cases direct sequencing of the *Rns* gene has resulted in mixed sequences, suggesting either a mixed culture of more than one *Acanthamoeba* strain or the presence of multiple alleles of this gene in a single *Acanthamoeba* strain. A previous study has established that multiple alleles in a single *Acanthamoeba* strain were possible. Here we examine an *Acanthamoeba* isolate from a keratitis case that produced mixed sequences when PCR products were directly sequenced. A single *Acanthamoeba* trophozoite was directly isolated from an agar plate to produce a clonal culture derived from this single troph. Following PCR amplification *Rns* products were cloned and sequenced. This produced two distinct sequences, indicating the presence of two alleles in this isolate. One allele, *Rns* 02-039/1, is identical to the *Rns* gene of *A.* sp. strain Galka. The second allele, *Rns* 02-039/2, has 99.7% similarity with the *Rns* from *A. castellanii* strain V042. Both of these isolates are genotype T4 based on *Rns* sequence analysis. Further, both are in sub-genotype T4a identified by mitochondrial *rns* analysis, and both are in clusters of identical sequences in *rns* analysis. These data suggest that transfer of alleles (genetic exchange) may be possible between closely related strains of *Acanthamoeba*. Specifically, the results suggest a possible transfer of one allele (02-039/2) after the divergence of *A.* sp. Galka from *A.* sp. 02-039.

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NEW INSIGHTS INTO INTRA-AMOEBOZOAN PHYLOGENY

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ABSTRACT

The phylogeny of the Amoebozoa has gained considerable interest in the recent years, which may largely also be traced back to the fact that several amoebozoans are implicated in human disease.

The aim of the current study was to make an approach to the intra-amoebozoan phylogeny. The 18S rDNA gene of 16 new amoebozoan isolates belonging to different taxa was sequenced and a cluster analysis was performed including various other amoebozoans of which sequence data are available. It was shown, that several amoebozoan genera, including *Acanthamoeba, Vannella, Mastigamoeba, Rhizamoeba, Hyperamoeba* and *Physarum* do not appear as monophyla. Moreover, in our study the myxogastrid mycetozoans formed a completely distinct lineage and did not group together with the dictyostelid mycetozoans.

Altogether, this study indicates, that several amoebozoan genera might need to be reviewed and once again points up the difficulties in intra-amoebozoan phylogeny and classification.

Key words: Amoebae, Lobosea, Mycetozoa, Archamoebae, molecular phylogeny.

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THE IDENTIFICATION OF VAHLKAMPFIID AMOEBAE BY ITS AND 5.8S rDNA SEQUENCING.

Johan F. De Jonckheere [1*] *and Susan Browm*[2]

ABSTRACT

During recent years there have been several investigations of human keratitis cases in which vahlkampfiids have been isolated, but in none of these cases was there sufficient evidence to conclude that these amoebae actually caused the infection (De Jonckheere 2003). Furthermore, in most cases, the vahlkampfiid isolates were not identified to the level of genus or species - unsurprisingly, as a few years ago it was shown that even genus identification based on morphology is unreliable in the family Vahlkampfiidae (Brown and De Jonckheere 1999). Based on SSU rDNA sequences, rearrangements in genus assignment were proposed. In the present study we investigated whether ITS1, 5.8S and ITS2 sequences could be used to differentiate and identify these vahlkampfiids, as demonstrated previously for the genus *Naegleria*.

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IDENTIFICATION OF 18S RIBOSOMAL DNA GENOTYPE OF *Acanthamoeba* FROM HUMAN WITH KERATITIS IN NORTH CHINA.

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ABSTRACT

Purpose This work is to identify the genotype of 18S ribosomal RNA gene (18S rDNA, *Rns*) of the *Acanthamoeba* strains isolated from the patients with keratits in northern China. **Methods** Genus specific primer JDP1 and JDP2 are used for the amplification of amplimer ASA.S1. With DNA PCR and sequencing, *Rns* genotypes were identified according to the DF3 sequences variance. **Results** Of all 26 DF3 sequences obtained from the 26 Acanthamoeba strains, 18 are unique (69.2%). Of the 18 unique sequences, 17 are *Rns* genotype T4 and 1 sequence is *Rns* genotype T3. **Conclusions** The majority of *Acanthamoeba* strains isolated from keratitis in North China are *Rns* genotype T4. This is in agreement with recent results in the literature.

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2D-PAGE PROTEIN PROFILES OF PATHOGENIC AND NON-PATHOGENIC *Naegleria* SPECIES.

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ABSTRACT

Naegleria fowleri, a causative agent of primary amoebic meningoencephalitis (PAM), is known to be morphologically indistinguishable from non-pathogenic *N. lovaniensis*. Their protein profiles by isoelectric focusing (IEF) separation were reported to be also quite the same. Recent technological progress of analytical methods makes it possible to compare proteins of closely related species by analyzing the spots into amino acid level after being separated by 2D-PAGE.

An Averaged gel of *N. fowleri* which ideally represents protein spot patterns common in the species was obtained from the 5 strains of this species, composed of 4 authentic strains (Nf66, KUL, LEE, 76/14/S3) and a Japanese isolate (KURUME), and detected 228 protein spots common in the species. Similarly, an Averaged gel of *N. lovaniensis* was prepared from 2 strains (Aq/9/1/45D, TS), and detected 246 common spots. The pairwise comparison of Averaged gels using computer-assisted spot matching showed that these two species exhibited marked diversity, with percentage of matching being 38.6% (88 common spots). Among 288 intra-specific common spots of *N. fowleri,* 53 spots were cut and analyzed N-terminal amino acid sequences. According to their sequences of about 20 residues, 11 protein spots were determined to be HSP-70, chaperone protein dnaK, three enzymes of carbohydrate metabolism and others. A 17.7 kDa protein (pI 6.9) was provisionally identified as Mp2CL5 membrane protein from *N. fowleri*.

Key words: *Naegleria fowleri*, *N. lovaniensis*, N-terminal amino acid sequence, proteomics.

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OPPORTUNISTIC INFECTIONS WITH *Acanthamoeba* SPP. IN MAN.

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ABSTRACT

Acanthamoeba spp. are opportunistic parasites causing infections in susceptible persons. Accidental colonization of respiratory tract of symptomless individuals has also been reported. The aim of our study was to detect *Acanthamoeba* spp. in BAL and sputum samples of immunocompromised persons with respiratory disorders to test the hypothesis that such patients may carry previously undetected infections with *Acanthamoeba* spp.

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RAPID IDENTIFICATION OF *Acanthamoeba* SPP. IN INFECTED TISSUES AND IN ENVIRONMENTAL SAMPLES USING CYTOCHEMICAL MARKER FOR CELLULOSE.

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ABSTRACT

The identification of *Acanthamoeba* spp. is primarily based on morphological features of the organism. Our objective was to develop a method, which would allow for the rapid and specific identification of *Acanthamoeba* spp. both, in clinical material and in environmental samples.

Amoebae strains used in the study were isolated from keratitis cases and from different water sources by filtration and subsequent cultivation on non-nutrient agar. Corneal samples from human *Acanthamoeba*-keratitis and tissues from experimentally infected mice were fixed in formalin and for sectioning embedded in paraffin or snap frozen. Dimers of cellulose-binding domains of *Trichoderma reesei* cellulase (D-CBD) obtained as a recombinant protein, were coupled to the fluorescent dye using Alexa Fluor® 568 Protein Labelling Kit and used in the direct immunofluorescence test for the detection of cysts of *Acanthamoeba*.

Our results showed that cellulose could be easily detected by immunofluorescence using conjugated D-CBD in the inner cyst wall of *Acanthamoeba* spp. The reference strains of *Acanthamoeba* spp. and all *Acanthamoeba* strains isolated from water and from keratitis patients gave positive reaction. All of *Naegleria* and *Hartmannella* isolates were negative in the test. Using the fluorescent conjugate of D-CBD parasites could be also demonstrated in mouse and human tissue sections.

We conclude that the D-CBD conjugate for direct staining of *Acanthamoeba* cysts is an alternative, potentially useful diagnostic tool, which allows for rapid and specific demonstration of parasites in both tissue sections and environmental amoebae.

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AGENTS OF AMOEBIC GILL DISEASE IN TURBOT, *Scophthalmus maximus* L.

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ABSTRACT

Amoebic gill disease (AGD), repeatedly declared the most serious disease affecting farmed salmonids, *Salmo salar* L. and *Oncorhynchus mykiss* (Walbaum, 1792) in the last two decades, was also diagnosed in turbot, *Scophthalmus maximus* (L.). Proper identification and systematic classification of AGD agent was set as an important step in the strategy employed to reduce significant stock losses both in salmonids and flatfishes. Comparative light and transmission electron microscopical studies of seven *Neoparamoeba* strains of different origin were not conclusive enough, since the only difference among the strains examined was found in the size of trophozoites. Consequently, the study was completed with molecular studies. Small subunit ribosomal RNA gene sequences were determined for five *Neoparamoeba* strains isolated from gills of turbots. Phylogenetic analyses revealed that sequences of two strains clustered with sequences of six (mostly environmental) strains determined previously as *N. pemaquidensis*. Three other strains were branching out of *N. pemaquidensis* and *N. aestuarina* clades suggesting that they could be representatives of a separate *Neoparamoeba* sp. This raises the question whether they are also primary agents of AGD. They can be as many other free-living amoebae only associated with lesions caused in the gills of *S. maximus* by *N. pemaquidensis*.

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PHAGOCYTOSIS OF *Acanthamoeba* SP. FROM AQUIFER OF THE VALLEY OF MEZQUITAL (STATE OF HIDALGO, MEXICO).

Silvana Pertuz Belloso [1], *Elizabeth Ramírez Flores* [1] *and Miroslav Macek* [1,2]

ABSTRACT

The occurrence of free-living amoebae has been reported from different types of aquatic environment but still, there is a lack of data showing their importance in the aquatic microbial loop. Even less is known on the feeding activity of these amoebae in the natural environment including the groundwater. Genus *Acanthamoeba* dominated within the amoebae isolated from underground waters in the Valley of Mezquital (State of Hidalgo, Mexico). The aim of the study was to estimate the phagocytosis of *Acanthamoeba* sp. (axenic environmental isolate), in micro-well plate experiments using fluorescently labelled bacteria (FLB) method. A dispersed bacterial prey (*Enterobacter aerogenes*) was applied at a concentration of 10^6 to 10^7 FLB ml^{-1}. Within the incubation (less than 1 h), *Acanthamoeba* sp. ingested in an average 2 to 4 FLB $cell^{-1}$, i.e., uptake rates of 6 to 54 FLB $cell^{-1}$ h^{-1} and vacuole forming rates 1 to 12 vac $cell^{-1}$ h^{-1} were reached. Generally, ingestion of FLB dropped due to an apparent saturation within the first 10 to 30 min of incubation with FLB; within 30 to 60 minutes, the number of observed vacuoles decreased as well, however, flocks of FLB were observed inside them. Numbers of FLB collected by amoebae from a dispersion varied too much to state a feeding rate valid for all populations. However, the data proved that acanthamoebae are able to collect a bacterial food from the dispersion at concentrations higher than 10^6 cells ml^{-1}.

Key words: amoebae, bacterivory, feeding, FLB.

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EUKARYOTIC ENDOSYMBIONTS OF *Neoparamoeba* SPP. ISOLATED FROM FISH.

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ABSTRACT

Light and electron microscopic observations on six strains of *Neoparamoeba* spp. isolated from gills of turbot, *Scophthalmus maximus* L., revealed in all studied strains the presence of so called "parasome" resembling symbiotic organism described previously as *Perkinsiella amoebae* Hollande, 1980 in trophozoites of *Janickina* spp. isolated from chaetognaths. (Similar organisms were recorded from 7 species belonging to 3 genera of the family Paramoebidae). Since comparative study of these organisms revealed their mutual similarity, we call the symbionts found in *Neoparamoeba* trophozoites *Perkinsiella amoebae*-like organisms (PLOs). The morphology of PLOs living together with *Neoparamoeba* trophozoites supported Hollande's hypothesis on their kinetoplastid origin but the first conclusive results were obtained in this study using marker genes. The recognition of euglenozoan spliced leader RNA (SL RNA) gene sequences in the genomic DNA of endosymbionts from *Neoparamoeba* strains together with aquisition of one SSU rRNA gene sequence, allowed us to specify the relationship of endosymbionts under study with kinetoplastids. Phylogenetic analyses of SSU rRNA gene sequence data currently available revealed close relationship of the first sequenced PLO with *Ichthyobodo necator*. Three types of the SL RNA gene sequences obtained from PLOs were congruent with phylogeny of their *Neoparamoeba* host strains.

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AMPHIZOIC AMOEBAE ISOLATED FROM FISH AS HOSTS OF OTHER ORGANISMS.

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ABSTRACT

Transmission electron microscopy routinely employed in the research of the genus and species composition of amoebae that infect freshwater and marine fishes expanded to the knowledge of organisms living in the cytoplasm of amoebae. After long period of axenization, *Acanthamoeba* trophozoites of strains isolated from liver of *Perca fluviatilis* L. and brain of *Leuciscus cephalus* (L.) hosted bacteria in the cytoplasm and trophozoites of the strain isolated from spleen of *Silurus glanis* L. contained virus-like particles. The cytoplasm of a newly described species of amphizoic amoeba, *Nuclearia pattersoni* isolated from gills of *Rutilus rutilus* L. contained rod-shaped bacteria, that on the basis of phylogenetic analyses of 16S rRNA gene sequences, were classified as *Rickettsia* sp. related to endosymbionts of leeches. Species of the genus *Labyrinthula* Cienkowski, 1867 was found in the cytoplasm of *Thecamoeba hilla* Schaeffer, 1926. Neither the effort to purify amoebae from this organisms, nor clonning procedure remove it from amoebae. The co-existence of both organisms sustainable for more than two years (the period of culturing) can be interpreted as stable type of symbiotic association.

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DIFFERENTIAL CYTOKINE GENE EXPRESSION ELICITED BY *Acanthamoeba* spp. AND *Balamuthia mandrillaris.*

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ABSTRACT

Acanthamoeba spp. and *Balamuthia mandrillaris* are opportunistic pathogens which are causative agents of granulomatous amebic encephalitis (GAE). In this study the highly pathogenic species *Acanthamoeba culbertsoni* was compared to *Balamuthia mandrillaris and Acanthamoeba castellanii* as to its capacity to elicit pro-inflammatory cytokine gene expression by neonatal rat microglia. A multiprobe RNase protection assay was utilized to study the induction of cytokine mRNA in primary microglial cells co-cultured with amebae. Scanning and transmission electron microscopy were used to examine the cytopathic effect of amebae on microglia. Microglia were capable of phagocytizing *A. castellanii.* In contrast, pathogenic *A. culbertsoni* and *B. mandrillaris* escaped the amebicidal activity of microglia. *A. culbertsoni* elicited a robust pro-inflammatory cytokine gene response by neonatal rat microglia *in vitro* as compared to *A. castellanii. B. mandrillaris* elicited a response intermediate to that of *A. culbertsoni* and *A. castellanii.* The preponderant cytokine elicited at the mRNA and protein levels was interleukin-1 beta (IL-β). Production of this cytokine was effected equally by intact *A. culbertsoni* amebae or *A. culbertsoni*-conditioned medium. These results suggest that *A. culbertsoni* secretes pro-inflammatory cytokine inducible factors. Furthermore, the preponderant elicitation of IL-1β by microglia articulates a mode by which the opportunistic amebae induce granuloma formation and cause pathology of GAE.

Keywords: *Acanthamoeba culbertsoni*, *Acanthamoeba castellanii*, *Balamuthia mandrillaris*, granulomatous amebic encephalitis, interleukin-1β, microglia, microglial cytokines.

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DETECTION OF ANTIBODIES TO *Acanthamoeba polyphaga* IN HUMAN SERUM, SALIVA AND COLOSTRUM.

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ABSTRACT

Studies related to the humoral and cellular mechanisms of the human mucosal immune system and how they may interact and eliminate *Acanthamoeba polyphaga* are lacking. Therefore, the aim of this study was to evaluate the serum and secretory antibody response to *Acanthamoeba* spp in healthy subjects. IgA antibodies to *A polyphaga* in colostrum of healthy women as well as in saliva and serum of healthy subject were analyzed by ELISA and western blot analysis. In serum, saliva and colostrum, we detected IgA antibodies that recognized several antigens of *A polyphaga*. IgA antibodies may avoid *Acanthamoeba* invasion. Anti-*Acanthamoeba polyphaga* IgA may participate in the resistance against the amebic invasion, probably by inhibiting the adherence of the trophozoites to corneal and nasal epithelial cells.

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BIOCHEMICAL CHARACTERIZATION AND COMPARISON OF PROTEINASES FROM TWO STRAINS OF *Acanthamoeba.*

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ABSTRACT

Acanthamoeba spp are free-living amoebae that may cause some pathologies in humans. Several authors have suggested that proteinases could be playing an important role in the pathogenesis of diseases. In this work we performed a partial biochemical characterization of *Acanthamoeba* proteinases in crude extracts and in conditioned medium, using 7.5 and 12% SDS-PAGE copolymerized with 0.1% of porcine skin gelatin as substrate. We found that 7.5% SDS-PAGE displays a better pattern of proteinases than at 12% SDS-PAGE. We could distinguish proteinases from 30 to 188 kDa in *Acanthamoeba castellanii* and from 34 to 144 kDa in *Acanthamoba polyphaga.* Another finding was that at temperature of 35°C, more proteinase activity was observed in both *Acanthamoeba* strains. Using different pH conditions we found high proteinase activity from pH 3.0 to 9.0 in crude extracts and in conditioned medium of both amoebic strains. Using different inhibitors, we determined that the most common proteinase type belongs to serine proteinase and secondly to cysteine proteinase group. Our results suggest that proteinase patterns are more complex than previously reported and differences between *A.castellanii* and *A. polyphaga* could be determined with better resolution using 7.5% SDS-PAGE copolymerized gelatin gels.

Key words: Acanthamoebiosis, SDS-PAGE copolymerized gels, cysteine proteinases, serin proteinases, proteinase inhibitors.

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TESTING FOR *Balamuthia* AMEBIC ENCEPHALITIS BY INDIRECT IMMUNOFLUORESCENCE.

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ABSTRACT

Purpose: This study presents results of screening serum samples from encephalitis patients in California by indirect immunofluorescence as a means of detecting *Balamuthia* amebic encephalitis. The effort is part of the California Encephalitis Project, which was initiated to provide intensive diagnostic testing to determine etiology of all types of encephalitis cases in the state.

Methods: Indirect immunofluorescence was used on acute and convalescent serum samples from encephalitis patients to determine titers of *Balamuthia* antibodies in the patients.

Results: Out of 213 serum samples tested within the program, three cases of *Balamuthia* amebic encephalitis were detected, all of which were fatal. Two other cases, not part of the program, were also diagnosed.. Necrotic brain tissue from one of the victims yielded a new clinical isolate of *Balamuthia* at necropsy.

Significance: These results indicate that balamuthiasis is a significant cause of mortality in the California population, and that the disease is under-reported in the population.

Key words: Amebic encephalitis, *Balamuthia.mandrillaris.*

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EFFICACY OF ANTI-NEOPLASTIC DRUGS AGAINST *Acanthamoeba.*

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ABSTRACT

Current drug therapy for *Acanthamoeba* keratitis is not always successful and late presenting cases are particularly difficult to treat. Therefore, new combination drug therapies are needed with enhanced activity to treat this painful, potentially blinding infection. This preliminary study was undertaken to determine the efficacy of three anti-cancer drugs against *Acanthamoeba*. MGBG, CHS 828 and hexadecyl-phosphocholine (HePC, Miltefosine) were tested individually or in combination with chlorhexidine, PHMB and propamidine. Sensitivity assays were performed over 48h in 96-well microtitre plates, using doubling dilutions of the drugs. MGBG had a trophozoiticidal concentration of 300µg/ml (1.2mM) and a cysticidal concentration of 1250µg/ml (4.6mM). The combination drugs had no effect on the cysticidal concentration of MGBG, but PHMB and propamidine may have an additive effect against trophozoites. CHS 828 [10 - 660µg/ml (27µM – 1.8mM)] caused trophozoites to round up, but had no effect on trophozoite or cyst viability. HePC killed both trophozoites and cysts at a concentration of 330µg/ml (0.8µM). Propamidine had an additive effect on HePC reducing the inhibitory concentration for trophozoites to 82µg/ml (0.2mM) and 165µg/ml (0.4mM) when added at high and low concentrations respectively. Synergy between HePC and chlorhexidine, PHMB or propamidine was found against cysts, being most active for chlorhexidine at 2µg/ml (minimum cysticidal concentration for HePC reduced to <10µg/ml). Further work is necessary to determine the potential of two of the anti-cancer drugs tested here, MGBG and HePC, as therapeutic agents for multi-drug treatment of infection by *Acanthamoeba*.

Key words : *Acanthamoeba*, MGBG, CHS 828, hexadecyl-phosphocholine, chlorhexidine, propamidine, PHMB.

INTRODUCTION

The life cycle of the ubiquitous free-living protozoa *Acanthamoeba* alternates between a

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AN *In vitro* ASSAY FOR TESTING CHEMOTHERAPEUTIC AGENTS FOR INHIBITION OF *Balamuthia mandrillaris* CYTOPATHOGENICITY.

Phiroze S. Tata[1], Elke Radam[1], Oliver Kayser[2] and Albrecht F. Kiderlen[1]

ABSTRACT

Balamuthia mandrillaris (Bm) was first described 1990 as an opportunistic causative agent of lethal granulomatous amebic encephalitis in man and other mammalian species (1). The mechanisms of pathogeneses, criteria for an early diagnosis and a reliable therapy remain unsolved. The *in vitro* data from Schuster (2) reveal sensitivity of Bm for some pharmaceuticals in terms of proliferation over long incubation periods. However, little is known regarding the amebicidal effect of drug action in the first 24 hours. As the most of the drugs are rapidly metabolized in the body as functionally inactive substances, the amebicidal drugs are preferable over amebastatic once.

In our study a panel of more than 40 established and novel pharmaceutical agents were tested for inhibition of the cytolytic activity of Bm. The assay proved useful as a highly sensitive, reliable and time-saving means of screening drugs for early effects on Bm.

The assay is based on the release of bacterial β-galactosidase (β-gal) as reporter enzyme from stably transfected murine mastocytoma P815 target cells. In intact cells, β-gal accumulates in the cytoplasm. Elevated concentrations of β-gal in the supernatant, measured as conversion of a luminescent substrate, indicate lysis and death of the cells.

Ballamuthia ameba are spontaneously cytopathic for eukaryotic cells. This can now easily be measured as β-gal release by P815 (β-gal) cultures. A drug that is toxic for this ameba is very likely to affect its cytolytic potency, either by direct kill or by physiological inhibition.

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THE OPPORTUNISTIC AMEBA *Balamuthia mandrillaris*, POSSIBLE PATHWAYS OF INFECTION.

Albrecht F. Kiderlen[1], Phiroze S. Tata[1], Ulrike Laube[1] and Klaus Janitschke[1]

ABSTRACT

Balamuthia mandrillaris was first described 1990 as an opportunistic causative agent of lethal granulomatous amebic encephalitis (GAE). Predispositions include general debilitated conditions, therapeutic or acquired immunosuppression (*e.g.* AIDS), and an immature or senescent immune system. In the murine model, infection is initiated by intranasal instillation of an ameba suspension. This procedure is based on the assumption, that natural infection follows either an olfactory-neurological or an olfactory-pulmonary-hematological route as shown for infections with *Naegleria* and *Acanthamoeba* respectively. The aim of this study is to describe and assess alternative routes of infection that might also be relevant following contact with *Balamuthia* contaminated environment.

Highly *Balamuthia*-susceptible BALB/c$^{scid/scid}$ mice (genetically defect for T- and B-cell maturation) were infected with *B. mandrillaris* organisms cultured on rat glioma as feeder cells. 1 x 10^4 trophozoites and cysts (ca. 10 %) per mouse were inoculated intranasally (i.n.), intratracheally (i.t.), intravenously (i.v.), or intracutaneously (i.c.); alternatively, 5 x 10^3 ameba/ mouse were given in drops onto the eyeballs (epiorbitally, e.o.). All pathways proved infectious, causing general signs of morbidity (weight loss, ruffled fur, prolonged crouching, neurological symptoms) and later death. The i.v.-infected group was the first to express symptoms, followed by those infected i.n., e.o., i.c. and i.t.. Histological examinations were performed to document the progress of the ameba from the site of infection to the brain. Especially the i.v. route led to dramatic pathological manifestations in most internal organs. Granted that little is known about the natural habitat this ameba, its infectious stages, routes of infection and infective numbers, these data do show that multiple routes of infection with *Balamuthia mandrillaris* are possible as are systemic infections with extracerebral manifestations. Multiple case reports on *Balamuthia* GAE mention prevailing skin lesions. These might now be reconsidered as one of the portals of entry for this pathogen.

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IDENTIFICATION OF *Acanthamoeba* FROM RECREATIONAL WATER BY POLYMERASE CHAIN REACTION TECHNIQUE.

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ABSTRACT

Previous studies have demonstrated that *Acanthamoeba* can cause several infections to the man as granulomatous amoebic encephalitis (GAE) and keratitis amoebic (KA), associated with the use of contact lenses. One of the problems associated with the detection of free-living amebas (FLA), from environment and from clinical samples is the time required to identify them, that in average is of one to three weeks. The purpose of this investigation was to compare the traditional method with PCR technique for the identification of pathogenic amebas from recreational water.

Samples (148) of springs, waterfalls, lagoons, and pools fed with thermal waters were obtained from 9 sites of the Huasteca zone of San Luis Potosi State, Mexico. The samples were processed by conventional laboratory methods to detect amebas of *Acanthamoeba* genus and at the same time were analyzed by PCR technique. To detect amebas belonging to the genera *Acanthamoeba* was used ($GP(P_2)$ primer, and to select the pathogenic amoeba of the genus was used Ac6 primer. In 30 water samples, the results obtained with the ($GP(P_2)$ coincided with the traditional method. In the case of the Ac6 primer, 25 samples coincided in the two methods. Comparing both identification methods, was observed that the technique of PCR results more adequate for identification of amebas of the genus *Acanthamoeba*.

Key words. *Acanthamoeba*, human pathogen, DNA sequence, PCR technique.

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DETECTION OF ANTIGENIC PROTEINS OF *Naegleria fowleri* BY SERIC ANTIBODIES OF INHABITANTS OF THE MEXICALI VALLEY, MEXICO.

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ABSTRACT

The Mexicali Valley, Baja California is a region in the northwest of Mexico irrigated by artificial shallow channels. During spring–summer, the environmental temperatures reach 55 °C. *Naegleria fowleri* (Nf) has been isolated in this region and also human cases of Primary Amebic Meningoencephalitis (PAM) have been reported. In order to detect antigenic proteins of *N. fowleri* by specific seric antibodies of inhabitants of the Mexicali Valley, sera samples of people that live in bordering zones to the irrigation channels were taking. The antibody seric levels of IgG anti-*N. fowleri* were evaluated by ELISA technique to 1:100 and 1:500 dilutions. Antigenic proteins patterns detected were recognized by this class of antibodies through the immunoblot technique. With the selected samples, adsorption assays were made using strains of reference of *Entamoeba histolytica* and *Acanthamoeba polyphaga* to eliminate antibodies of cross-reaction. High antibodies levels of IgG anti-Nf were detected. By immunoblot, antigenic proteins of *N. fowleri* with weights molecular between 120 and 6.5 kDa were detected. After adsorption assays, the relevant antigenic proteins of Nf, detected by seric antibodies of inhabitants of Mexicali Valley are: 95, 36 y 6.5 kDa. This proteins might play an important role in the mechanisms of invation of Nf or to be useful for diagnosis.

Key words: IgG, free-living amebas, artificial channels.

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NO PRODUCTION AND IMMUNOREACTIVITY WITH ANTI-NITRIC OXIDE SYNTHASES 1-2-3 ANTIBODIES BY *Naegleria fowleri.*

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ABSTRACT

Nitric oxide (NO) is a multifunctional agent, which serves as a key signaling molecule in physiological processes such as host defense, neuronal communication and regulation of the vascular tone. Nitric oxide synthases (NOSs) are the enzymes responsible for synthesis of Nitric oxide from L- arginine. The free-living ameba *Naeglaeria fowleri* can produce a rapid fatal infection disease named Primary Amebic Meningoencephalitis (PAM), but their pathophysiological mechanisms are largely unknown. Several reports have shown that NO has a cytotoxic effect against a variety of parasites. However, *N. fowleri* trophozoites are highly pathogenic since we have observed that they can kill activated peritoneal macrophages expressing inducible NOS suggesting that throphozoites may present a high resistance degree to NO toxicity. The aim of this work was to determine if *N. fowleri* can produce NO. Besides we analyzed whether antibodies anti the three mammalian NOS isoforms (neuronal, inducible and endothelial) presented immunoreactivity to *N. fowleri* trophozoites. Present results indicate that *N. fowleri* trophozoites can produce NO. Besides Western blot evidences showing that *N. fowleri* trophozoites contains proteins that share epitopes with the three described mammalian NOS, but whose relative molecular weights are different to those described for mammalian NOS; suggesting that *N. fowleri* may contain undescribed NOS isoforms.

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CROSS-REACTION BETWEEN *Naegleria fowleri, Acanthamoeba culbertsoni* AND *Entamoeba histolytica.*

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ABSTRACT

The interest for pathogenic free-living amebas (FLA) has been increasing due to the knowledge that they can cause human disease and death. *Naegleria fowleri* produces a fulminant infection called Primary Amebic Meningoencephalitis (PAM), *Acanthamoeba* spp. can cause Granulomatous Amebic Encephalitis (GAE), *Acanthamoeba* keratitis (AK), paranasal sinusitis, pneumonitis, skin nodules and ulcers.

The purpose of this work was to determine whether cross reactivity exists among antigens of *N. fowleri, Acanthamoeba culbertsoni* and *Entamoeba histolytica* using antibodies against each of the three amebas.

Extracts of *N. fowleri, A. culbertsoni* and *E. histolytica* were obtained by sonication and the pattern of proteins was analyzed by electrophoresis. Specific antibodies against each ameba were obtaines by immunization of rabbit with amebic extracts. The recognition of specific antibodies was analyzed by immunoblots. In order to try to identify *N. fowleri* specific antigens that may be useful for diagnosis, adsorptions of antibodies anti-*N. fowleri* with fixed extracts of the three strains were performed to eliminate cross-reactions and identify specific antigens of each species. By immunoblots, it was found that cross-reactions exist among *N. fowleri, A. culbertsoni* and *E. histolytica*, being stronger this reaction between *N. fowleri* and *E. histolytica.*

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ABSTRACT

The interest for pathogenic free-living amebas (FLA) has been increasing due to the knowledge that they can cause human disease and death. *Naegleria fowleri* produces a fulminant infection called Primary Amebic Meningoencephalitis (PAM), *Acanthamoeba* spp. can cause Granulomatous Amebic Encephalitis (GAE), *Acanthamoeba* keratitis (AK), paranasal sinusitis, pneumonitis, skin nodules and ulcers.

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PRODUCTION OF MONOCLONAL ANTIBODIES TO *Naegleria fowleri*, AGENT OF PRIMARY AMEBIC MENINGOENCEPHALITIS.

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ABSTRACT

Monoclonal antibodies (Mabs) to *Naegleria fowleri,* the etiologic agent of primary amebic meningoencephalitis (PAM), where produced. The class and subclass of the produced antibodies, was determinated, using the ELISA test. The antibody activity to antigenic determinants presents in the cell membrane of *N. fowleri* and *Naegleria lovaniensis* was probed using flow citometry. This technique, was used in order to probe if *N. lovaniensis* share membrane's antigenic determinants with *N. fowleri.* Twenty one Mabs reacted intensely with *N. fowleri* but just only four of them conserved the stability to produce antibodies after time. Three of them resulted be class M immunoglobulin, one of them IgG and the other IgG3. In the FAC's test, the Mabs reacted with antigenic determinants presents in cell membrane of *N. fowleri* and four of them emitted fluorescence. Only one of the Mabs reacted both, with *N. lovaniensis* and *N. fowleri* and emitted fluorescence, indicating that these two species share antigenic determinants and then the Mab who was positive to flow citometry test had across reaction with *N. lovaniensis.*

Keywords: Antigenic determinants, clones, ELISA, hybrid cells.

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AMOEBAE OF GENUS *Acanthamoeba* AS VECTORS OF PATHOGENIC BACTERIA.

E. Hadas [1], M. Derda [1], J. Winiecka-Krusnell [2] and A. Sułek [1]

ABSTRACT

The significance of *Acanthamoeba* for maintaining and transmission of human pathogens is not well recognized. The aim of our study was to evaluate the association of potentially pathogenic *Acanthamoeba* spp. with bacteria in strains recently isolated from Malta lake near the city of Poznan, Poland. Most of the isolated amoebae belonged to species *A. castellanii* and *A. rhysodes*. The majority of isolates were pathogenic for mice and parasites could be recovered from their brains and lungs. In cases when amoebae could not be recovered from autopsy material histopathological analysis showed changes in tissues indicating bacterial infection. We found that approximately 50% of isolates were associated with bacteria e.g. *Proteus* sp., *Clostridium perfrigenus, Escherichia coli, Salmonella* sp., *Legionella pneumophila* and other species. The presence of bacteria residing inside free-living amoebae represents a challenge in terms of disease control and sanitation of contaminated water sources.

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DEVELOPMENT OF A TAQMAN REAL-TIME PCR ASSAY FOR THE DETECTION OF THE HUMAN PATHOGEN *Naegleria fowleri.*

Jonas Behets [1]*, *Lieve Verelst* [2] *and Frans Ollevier* [1]

ABSTRACT

The free-living amoebae of the genus *Naegleria* are widely distributed in humid habitats. They include the thermophilic *Naegleria fowleri*, the causative agent of primary amoebic meningoencephalitis (PAME) in humans. Prevention of PAME by monitoring environmental sites upon the presence of *N. fowleri* is therefore crucial. Hereto a rapid and specific quantitative detection method is urgently needed.

Quantitative detection methods (MPN) using traditional cultures followed by protein-electrophoresis, ELISA or PCR are time consuming (more than 5 days) and not very accurate. The aim of this study was to develop a rapid, sensitive and specific real-time PCR technique to detect *Naegleria fowleri* in cooling water samples within 24 hours.

In this study cooling water samples spiked with *N. lovaniensis* (a close relative to *N. fowleri*) were concentrated by filtration. Thereafter DNA extraction and neutralisation of inhibitors was performed, followed by Taqman real-time PCR. Results indicate that we were able to detect and semi-quantify *N. lovaniensis* in one day with real-time PCR and that this method can be used for the detection of *N. fowleri*.

Keywords : *N. lovaniensis, N. fowleri, real-time PCR*

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REAL TIME MONITORING OF *N. fowleri* IN POWER PLANT COOLING SYSTEM WATER.

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ABSTRACT

Naegleria fowleri is the causative agent of primary amoebic Meningoencephalitis, a disease of the central nervous system which is rapidly fatal. Because the presence of *Naegleria fowleri* is promoted by the warming of cooling water in nuclear power plants, it is necessary to monitor for the pathogenic micro-organism on a daily basis in summer. A new method for testing for the pathogenic species has been developed from two advanced technologies, immunofluorescence and Chem*Scan*™ solid phase cytometry, enabling a reduction in the time needed to confirm its presence.

To evaluate the benefits of the tool for monitoring of *N.fowleri* concentrations, a comparison was made of the counts obtained with cultures and by means of solid phase cytometry, on 87 samples of water taken from power plant cooling systems.

The two methods do not differ significantly in view of the uncertainties linked to the two methods of microbiological measurement.

The results of this initial validation phase indicate that it is possible to quantify the number of amoebae present in cooling systems in 3 hours, with a mean detection threshold of 200 amoebae/l in cooling system water treated with monochloramine.

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