Free Living Amoebae: Emerging Pathogens under the Radar

Dr Jacob Lorenzo Morales
Ramón y Cajal Tenure Track Researcher
IUETSPC-ULL

Free Living Amoebae

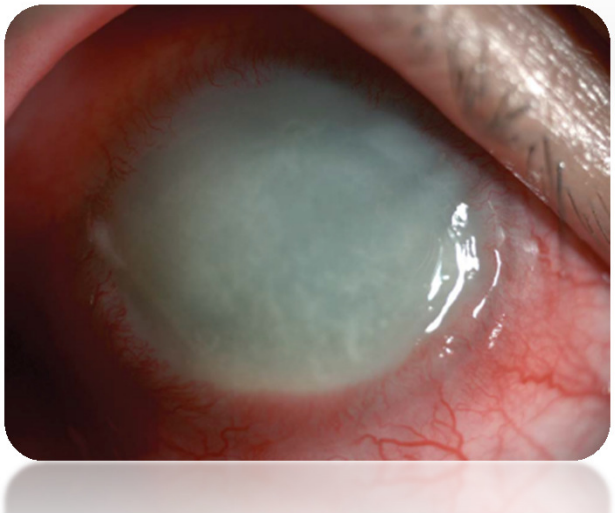
- Emerging Parasitic Protozoa.
- Life Cycle.
  - Trophozoite.
  - **Cyst**.
- Species/Genera:
  - *Naegleria fowleri*
  - *Balamuthia mandrillaris*
  - *Sappinia diploidea/pedata*
  - *Acanthamoeba* spp.



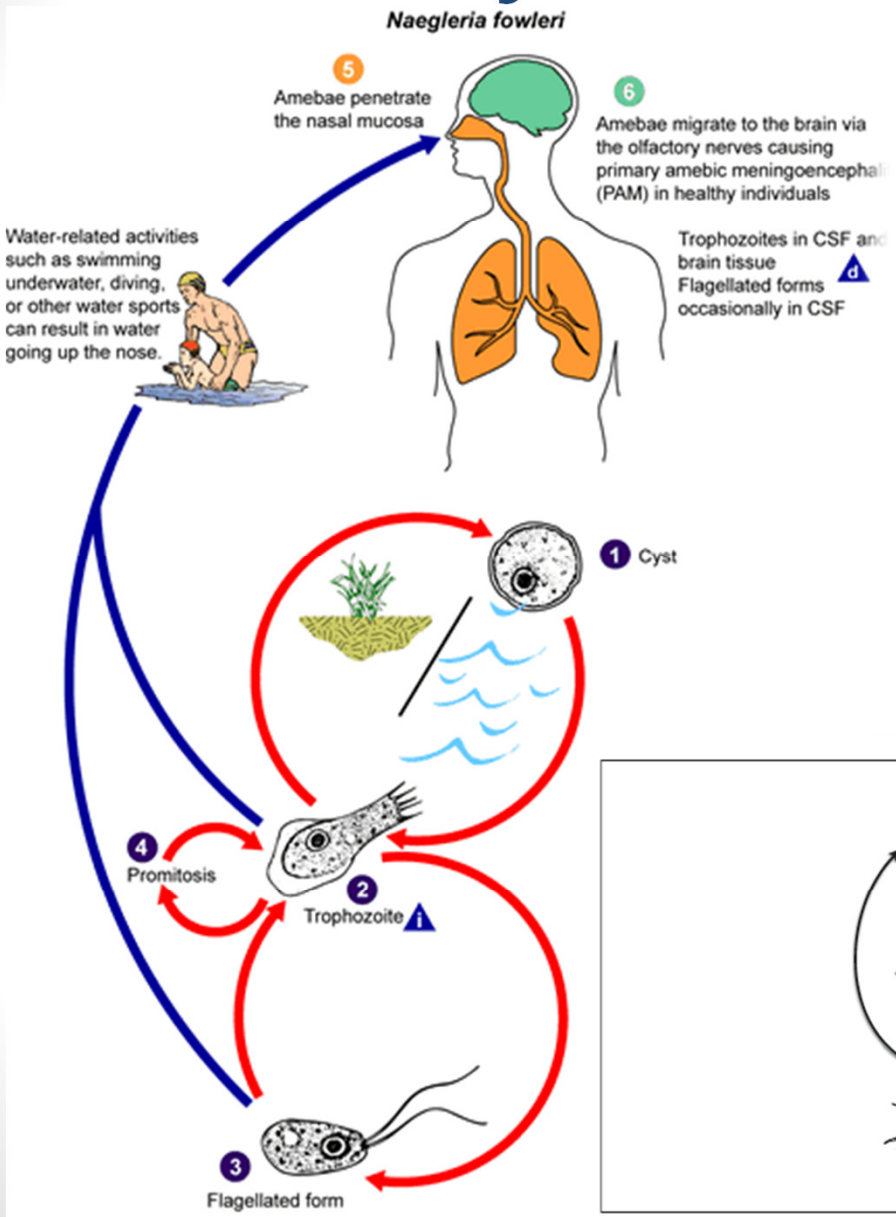
Pathology

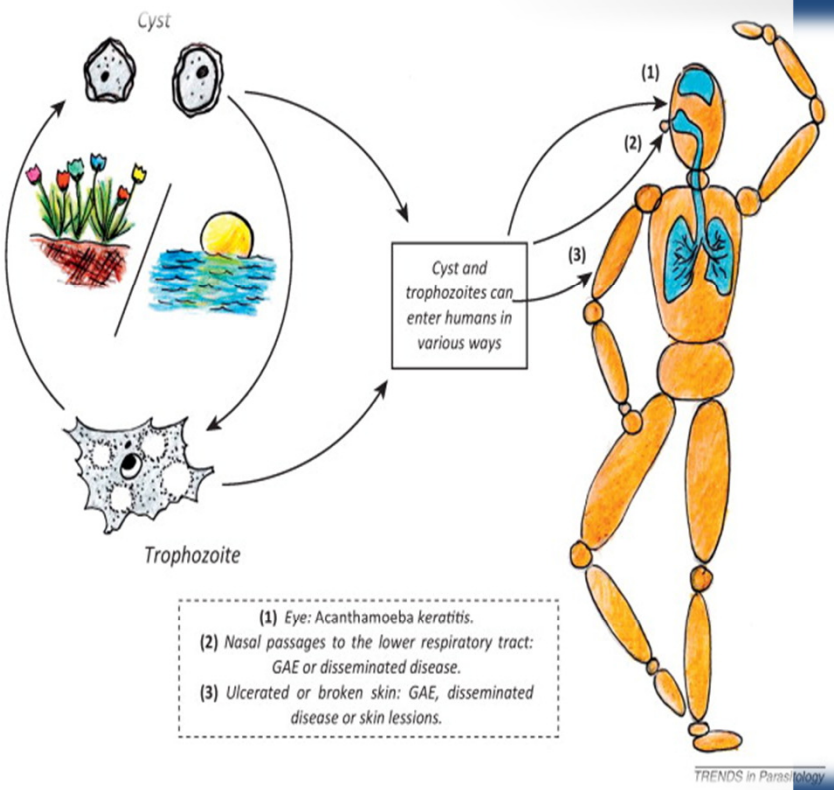
- Encephalitis
  - Fatal in more than 90% of cases
- Keratitis
  - *Acanthamoeba* spp.
  - Issues:
    - Diagnosis
    - Therapy

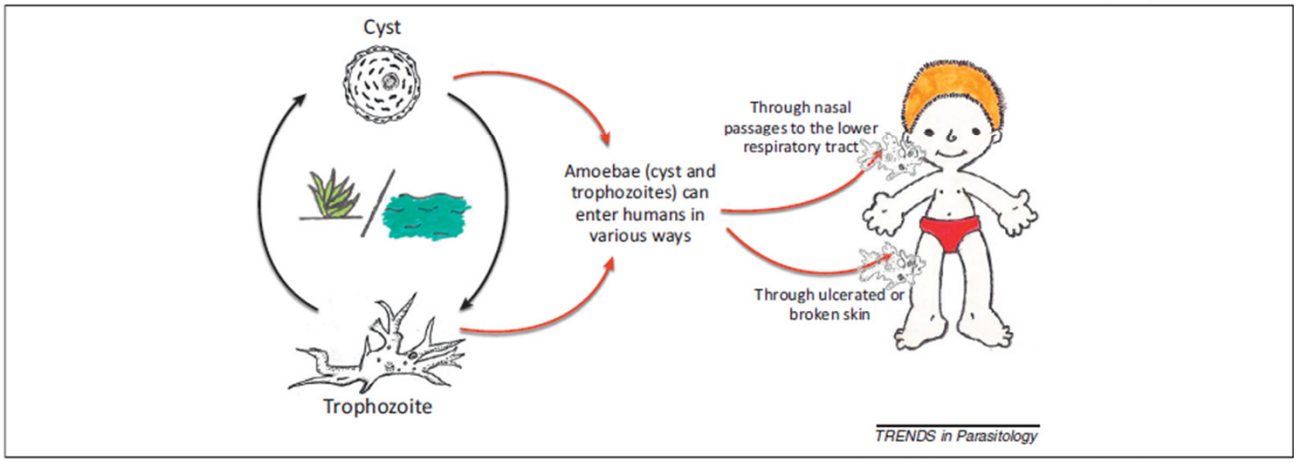




FLA life cycle







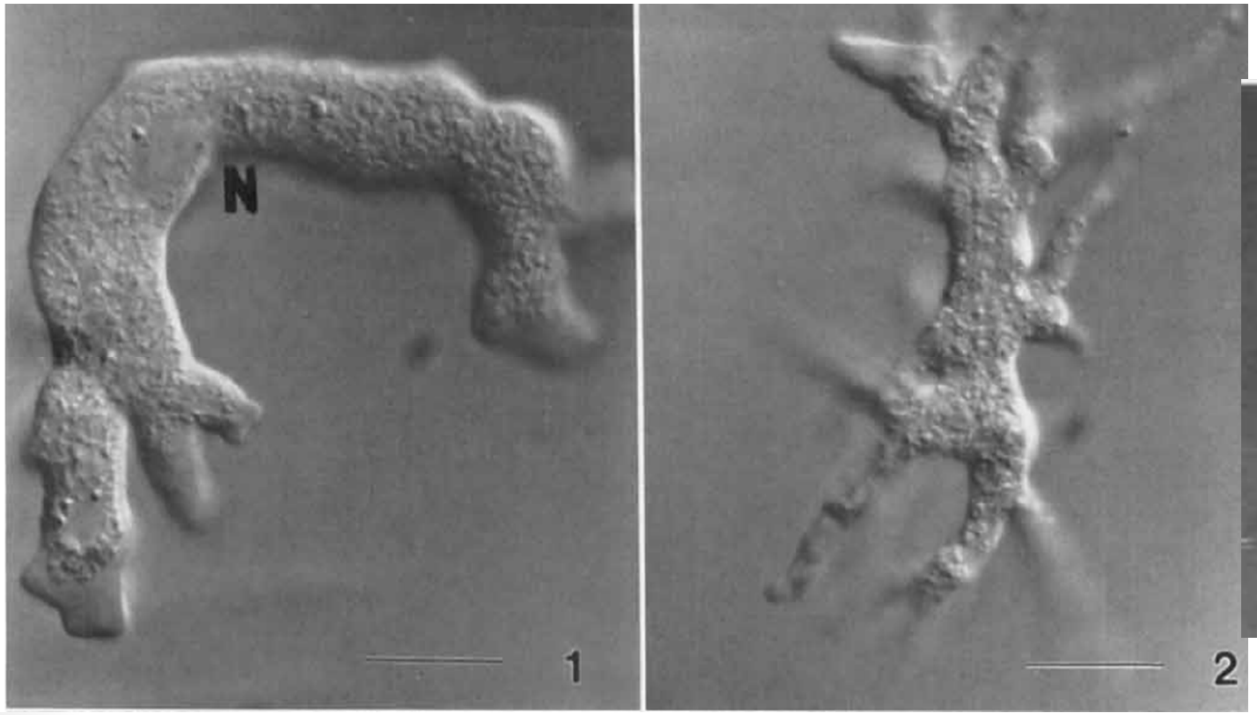
Universidad de La Laguna

J. Euk. Microbiol., 40(4), 1993, pp. 504–514
© 1993 by the Society of Protozoologists

Balamuthia mandrillaris, N. G., N. Sp., Agent of Amebic Meningoencephalitis in Humans and Other Animals

GOVINDA S. VISVESVARA,*,† FREDERICK L. SCHUSTER, and A. JULIO MARTINEZ*****

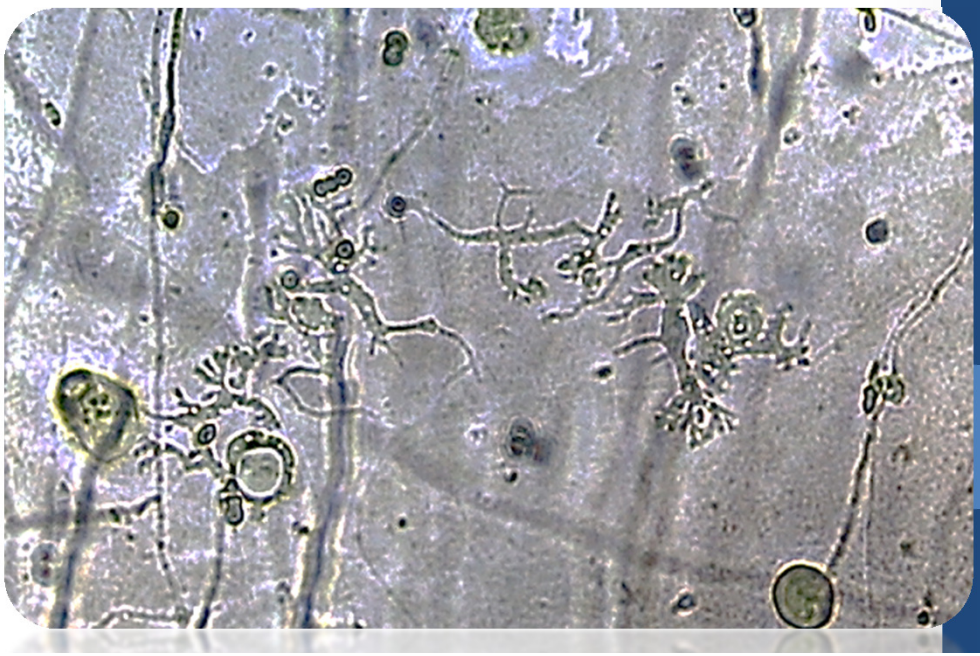
**Parasitic Diseases Branch, M.S.-F/13, Centers for Disease Control and Prevention, 4770 Buford Highway NE, Atlanta, Georgia 30341-3724,*
***Department of Biology, Brooklyn College, New York, New York 11210, and*
****Department of Pathology, Presbyterian Hospital and University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15231*





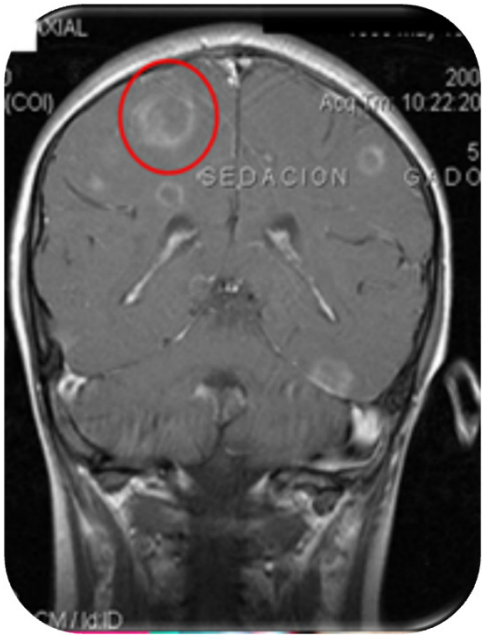
Balamuthia mandrillaris

- More than 200 reported cases worldwide
- Most affected countries so far → USA, Peru, Mexico
- Diagnosis→ PCR/IF *postmortem*
- Therapy!



B. Mandrillaris Therapy

- Combinations of miltefosine, pentamidine, azitromicine, fluconazole, fluorocitosine, albendazole and anfotericin B
- 10 survivors reported so far

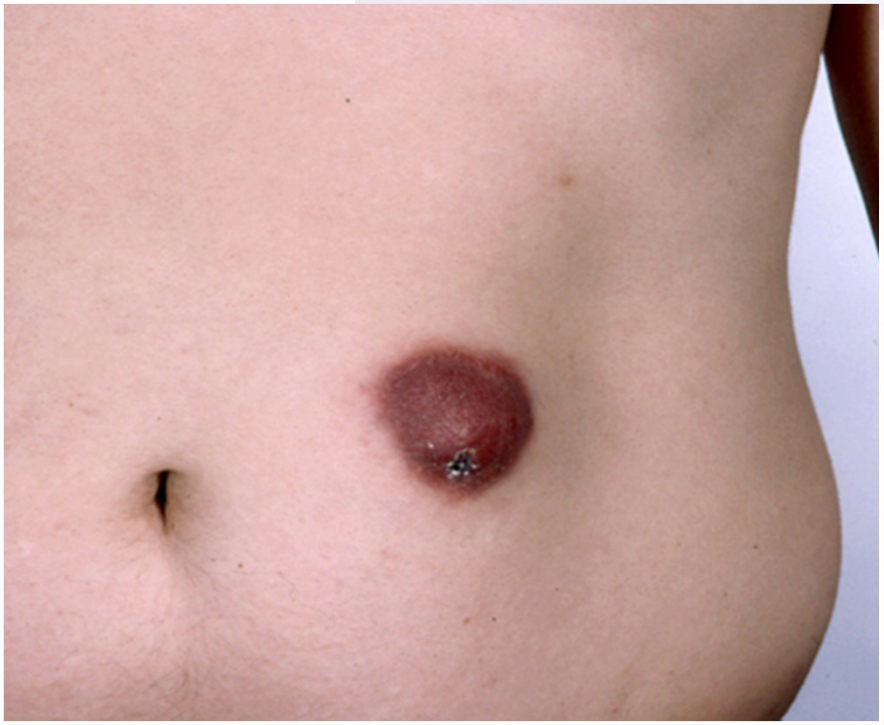




Balamuthia mandrillaris- Reported cases

- First reported isolation – brain tissue of a female mandrill in San Diego Wild Animal Park in 1986
- Since then more than 200 human cases have been reported
- Isolated from soil and dust from USA, Peru, Mexico, Iran, Costa Rica and Guinea-Bissau
- Environmental isolation reports are very low, why???







BAE in skin (Khan, 2006)

Clinical presentation in Peru



Bravo F G, Cabrera J, Gotuzzo E. Cutaneous manifestations of infection by free living amebas, with special emphasis on *Balamuthia mandrillaris*. En: Tyring SK, Lupi O, Henage UR, eds. Tropical Dermatology. Philadelphia: Churchill Livingstone; 2006: 49-56





Figure 6.3 Typical progression of the lesion, infiltrating all facial tissue.

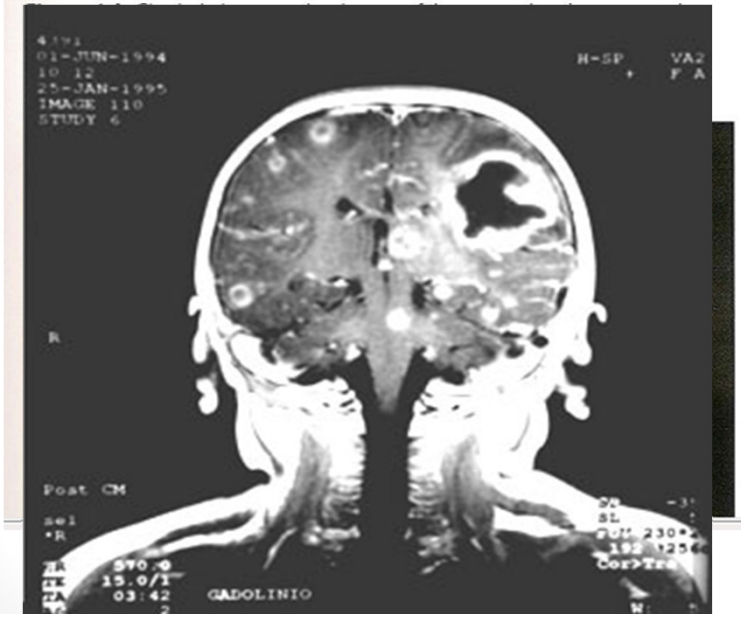




Figure 6.4 Lesion on the chest, excised after a partial response to medical therapy.



Environment of
BM in Piura - Perú





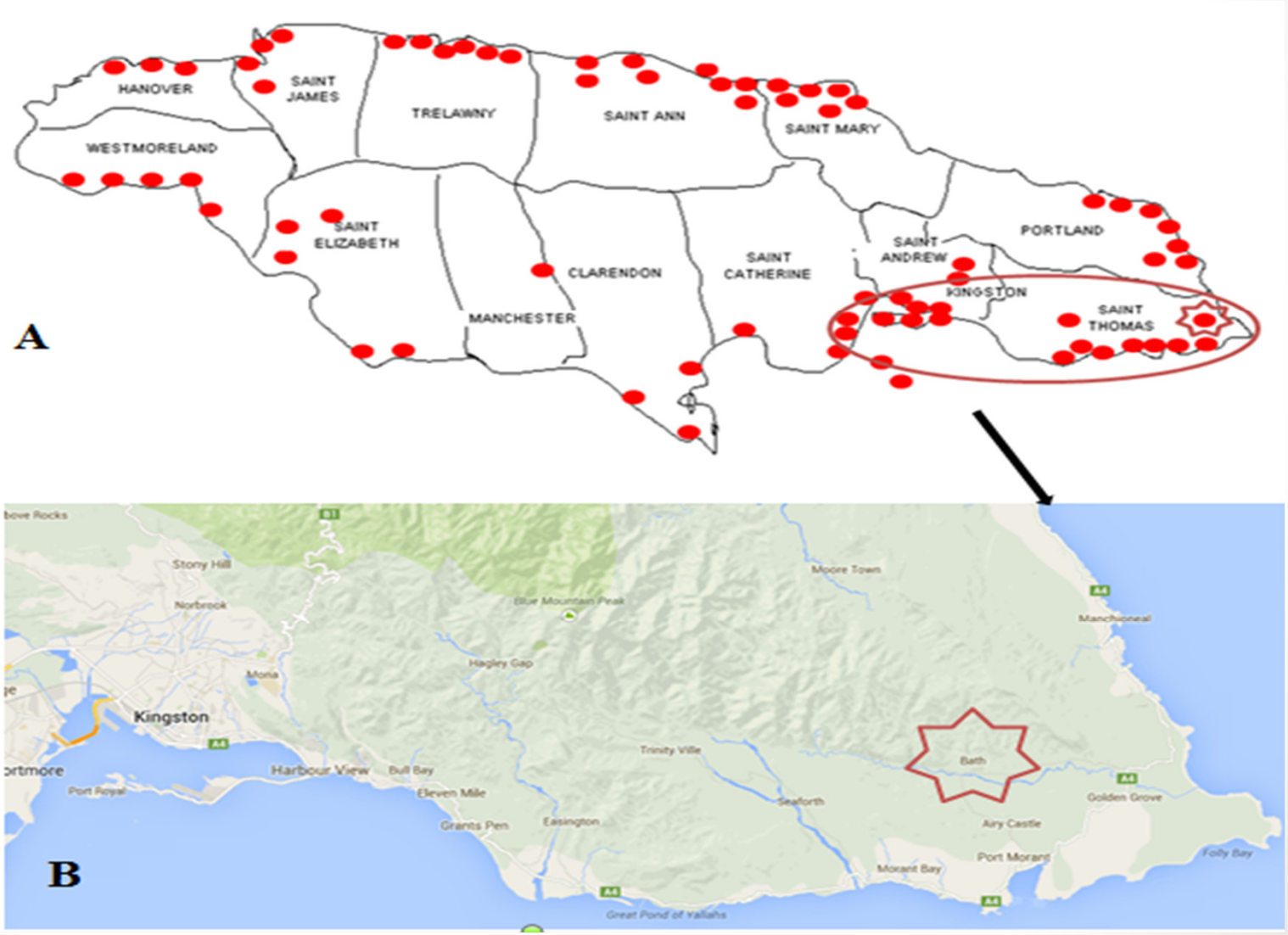






Jamaica

Sampling Site- Jamaica



Results

- Isolated from a single soil sample from a mineral spring in the parish of St. Thomas
- Was not isolated from mineral spring water
- PCR product of the isolate showed sequence homology of 98% compared to other *B. mandrillaris* sequences in Genbank

Balamuthia mandrillaris- Jamaica





- Mud bath- a common practice at the site

Balamuthia mandrillaris- Jamaica





- A resident being given a warm water bath and a tourist sun bathing at the site



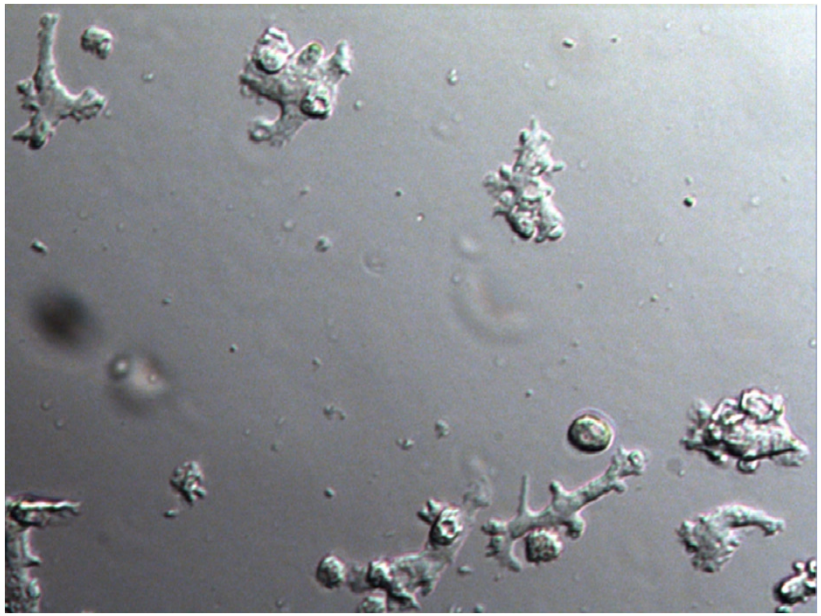


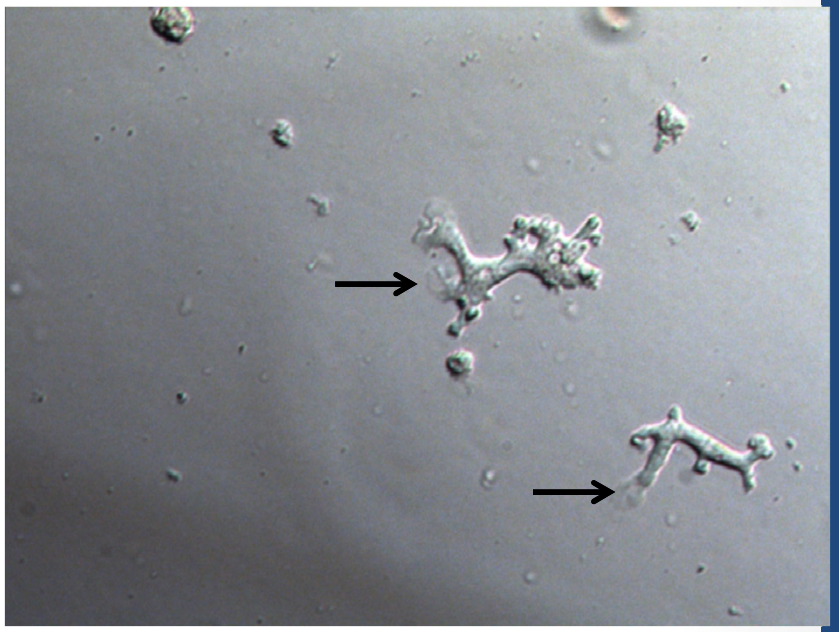


- Healing of skin lesions with combination mud and spring water

Balamuthia mandrillaris- Jamaica

- Trophozoites of *B. mandrillaris* isolated on NNA plates seeded with heat killed *E. coli*





Balamuthia mandrillaris

Balamuthia mandrillaris

- Although the amoeba was not isolated from the mineral spring water it was isolated from the soil (mud)
- Practices are potential high risk for infection
  - the mud bath business conducted at the site of the mineral spring which includes the application of mud to the skin for a few minutes before being washed off with spring water
  - provide direct entry portals for the amoebae through skin lesions

Conclusions

Epidemiol. Infect., Page 1 of 4. © Cambridge University Press 2014
doi:10.1017/S0950268814002842

SHORT REPORT
Balamuthia mandrillaris therapeutic mud bath in Jamaica

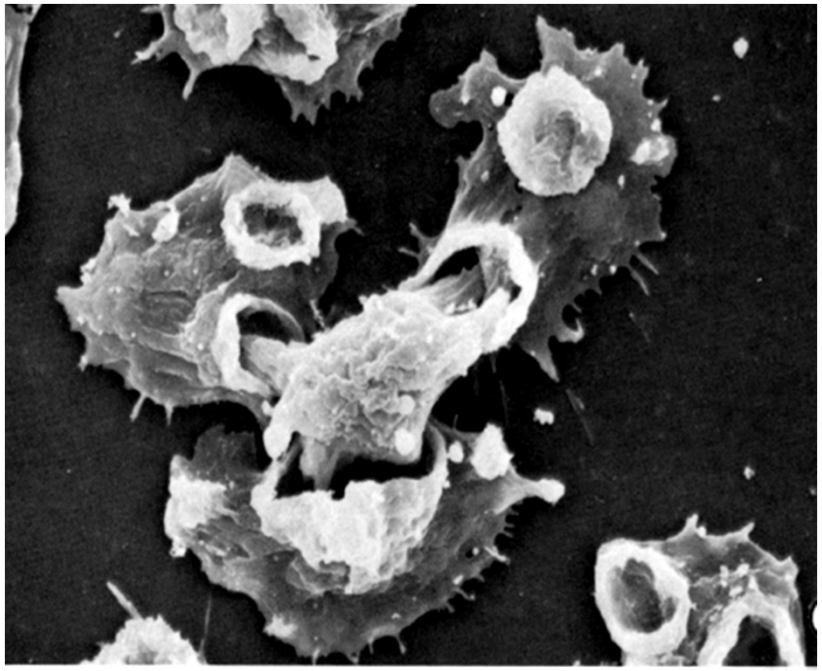
C. D. TODD[1,2], M. REYES-BATLLE[1], J. E. PIÑERO[1], E. MARTÍNEZ-CARRETERO[1], B. VALLADARES[1], J. F. LINDO[2] AND J. LORENZO-MORALES[1]*

[1] *University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, La Laguna, Tenerife, Canary Islands, Spain*
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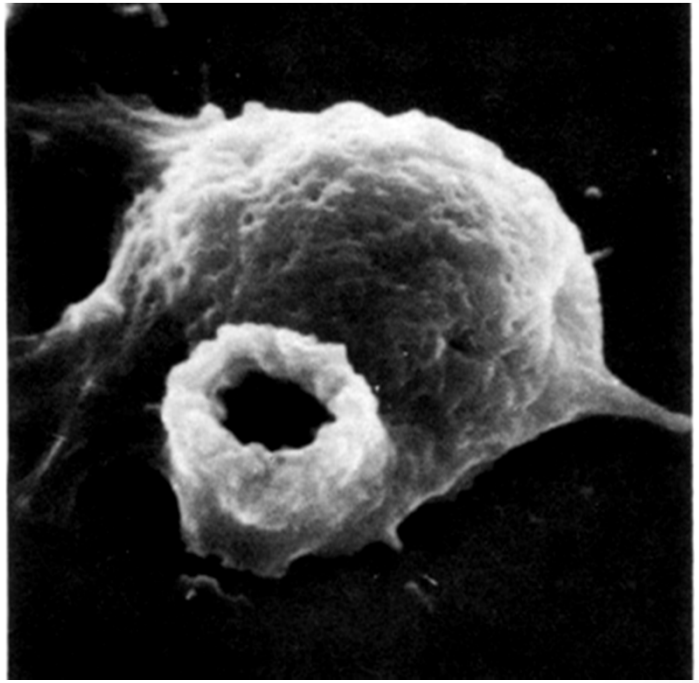
Received 29 July 2014; Final revision 2 September 2014; Accepted 30 September 2014


Dear Balamuthia,
please don't eat my brain

Naegleria fowleri



Appl Environ Microbiol. 1984 Jan;47(1):12-4.

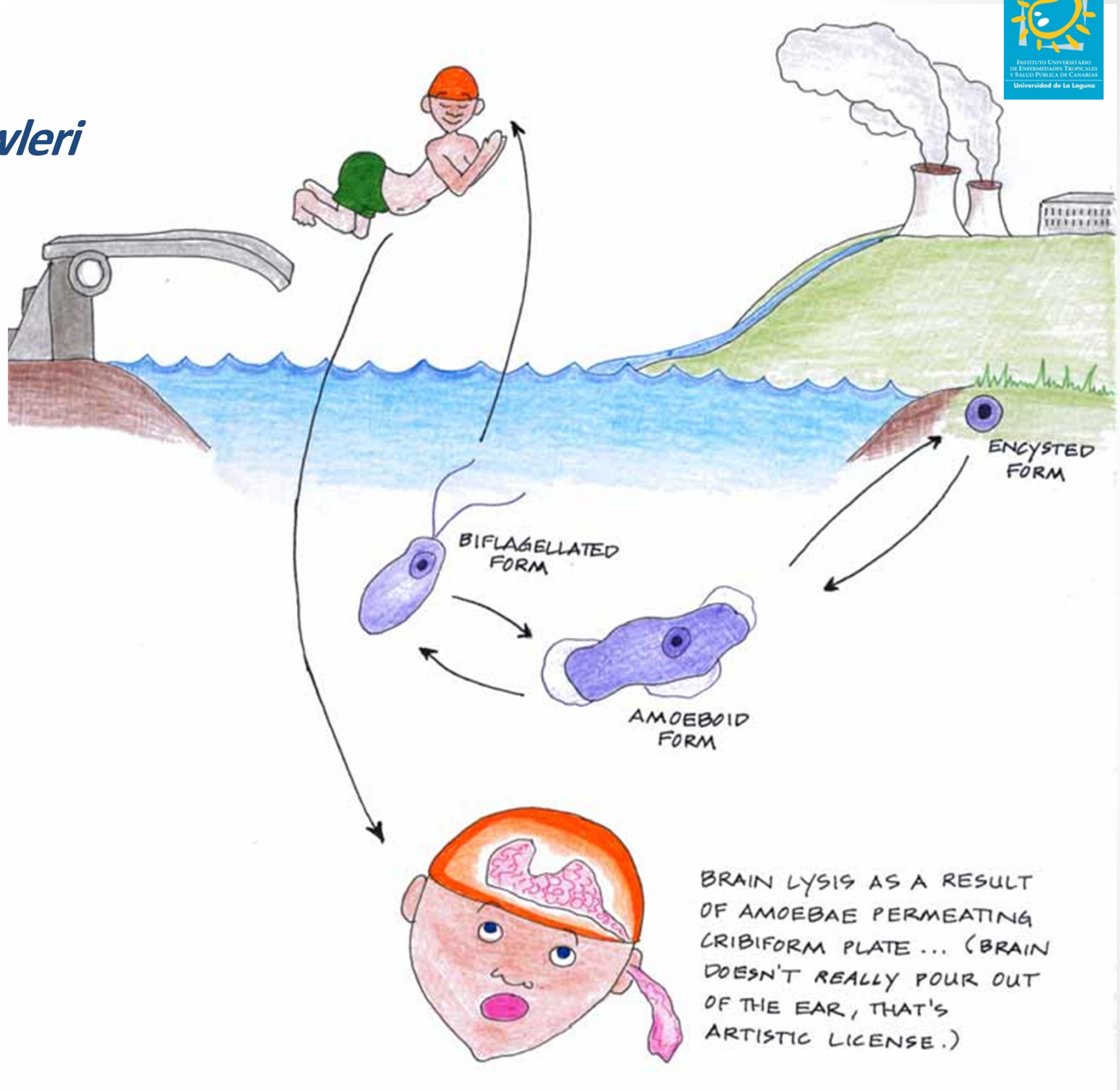


Infect Immun. 1983 Jun;40(3):1214-7.

Naegleria fowleri

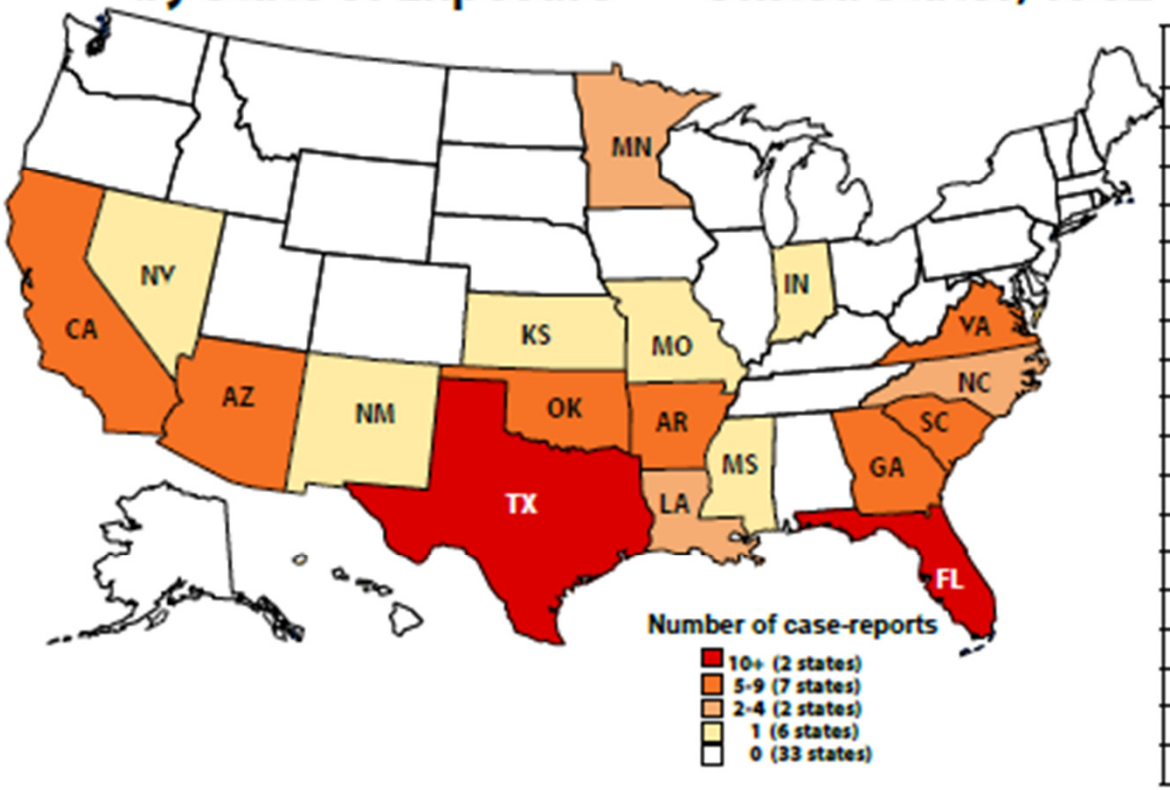
- *Naegleria* is widely distributed in warm water bodies → lakes, pools
- Only 1 pathogenic species→ *N. fowleri*
- Causative agent of PAM (Primary Amoebic Meningoencephalitis)
- Rare but lethal brain infection:
- From 1998 a 2007, 33 reported cases in USA (31 recreational water bodies and 2 in hot springs)
- Until 2007, 300 reported cases worldwide, 7 survivors
- From 2007-2015 → Higher incidence????

Naegleria fowleri



PAM

Number of Case-reports of Primary Amebic Meningoencephalitis Caused by *Naegleria fowleri* (N=132) by State of Exposure*— United States, 1962–2013



State (Abbreviation)	# of Cases
Arizona (AZ)	7
Arkansas (AR)	6
California (CA)	7
Florida (FL)	34
Georgia (GA)	5
Indiana	1
Kansas (KS)	1
Louisiana (LA)	4
Minnesota (MN)	2
Mississippi (MS)	1
Missouri (MO)	1
Nevada (NV)	1
New Mexico (NM)	1
North Carolina (NC)	4
Oklahoma (OK)	6
South Carolina (SC)	7
Texas (TX)	32
Virginia (VA)	7

- State of exposure unknown for 4 cases.
- Does not include one case from USVI.

2002

Deadly meningitis, double heartbreak

Pair of unrelated Valley 5-year-olds die

By Judd Slivka, Monica Mendoza and Maggie Galehouse
The Arizona Republic
Oct. 17, 2002

- Arizona kids both were reported to play under the water while having a bath at home.
- Water systems in the area were positive for NF
- The isolated strains from autopsies were 100% identifical to the one isolated from the water systems.

Summer 2011-
Winter 2012



How the problem was solved?



January 2012→ Neti pot



y MICHELLE CASTILLOCBS NEWSAugust 23, 2012, 3:37 PM

Tap water in neti pots behind two brain-eating amoeba deaths in 2011, investigation finds



A woman using a neti pot to clear her sinuses. **FDA**

- *Impl* *→ CDC*
  and
- *Fata* *ssible*
  Expo *fect Dis.*
  cir9(
- Loca

U.S.

La. health officials investigating how brain-eating amoeba got into water supply outside New Orleans, killing little boy

After 4-year-old boy dies from contaminated water, Louisiana authorities investigating, flushing water supply with chlorine. Water is safe, they say, but don't get it up your nose.

BY DEBORAH BASTINGS / NEW YORK DAILY NEWS

MONDAY, SEPTEMBER 16, 2013, 5:05 PM

101 17 0



Drake Smith Jr. ... eating amoeba ...

ideo: After CDC testing, a second Louisiana parish confirms that a brain-eating amoeba has been ound in their water supply. KTAL's Nikki Henderson reports.

Louisiana health officials have advised 82 of the stateís water systems to step up levels of disinfection to make sure theyíre killing off a deadly amoeba that killed a boy last month.

The amoeba, Naegleria fowleri, was found in a second water system this week, and itís possible water systems need to boost levels of the chlorine-based disinfectant they are using, the state department of health and hospitals says.

2013

CNN

... Middle East | Business | W...

...ek



Mom: Speak up to doctors

STORY HIGHLIGHTS

Only two patients had been known to survive in the last half-century.

"I've been in the hospital a long time," she says

Amoeba causes brain infection, nearly always fatal, according to the CDC

Parasite generally found in people who swim in warm fresh water

(CNN) -- By nearly all accounts, 12-year-old Kali Hardig should not be alive.

During a swim at a water park in July, she contracted parasitic meningitis, a rare infection caused by brain-eating amoebas that has a survival rate less than 1%.

Only two patients had been known to survive in the last half-century.

But Kali is a fighter and, against all odds, she made it. Wednesday, after nearly seven weeks, she finally left the hospital.

"It's awesome to be home," Kali told CNN's Kate Bolduan on "New Day" Thursday morning.

2014

- USA → 3 cases in Louisianna and 1 child in Florida. July 2014.
- Pakistan → 94 deaths reported







2014

- anti-*Brain eating Amoebae champaign MINNESOTTA*



Pakistan



DISPATCHES

Primary Amebic Meningoencephalitis Caused by *Naegleria fowleri*, Karachi, Pakistan

Sadia Shakoor, Mohammad Asim Beg, Syed Faisal Mahmood, Rebecca Bandea, Rama Sriram, Fatima Noman, Farheen Ali, Govinda S. Visvesvara, and Afia Zafar

We report 13 cases of *Naegleria fowleri* primary amebic meningoencephalitis in persons in Karachi, Pakistan, who had no history of aquatic activities. Infection likely occurred through ablution with tap water. An increase in primary amebic meningoencephalitis cases may be attributed to rising temperatures, reduced levels of chlorine in potable water, or deteriorating water distribution systems.

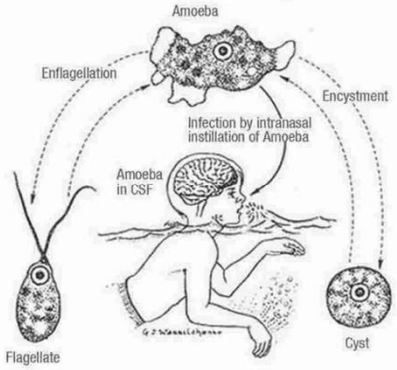
2014

GOVERNMENT OF SINDH
HEALTH DEPARTMENT

PUBLIC AWARENESS MESSAGE

Naegleria Fowleri is a deadly disease which has NO cure but is 100% preventable.

Naegleria Fowleri, (Brain eating Amoeba) that lives naturally in most warm fresh waterways including lakes, rivers, ponds, hot springs, soil and swimming pools that are poorly maintained.(Minimally or un-Chlorinated). It infects people by entering the body through the nose during swimming or diving in warm fresh water places and travels to the brain where it destroys the brain tissues.





SIGNS AND SYMPTOMS OF THE INFECTION INCLUDE:

- Severe frontal headache
- Stiff neck
- Fever
- Seizures
- Nausea
- Altered mental status
- Vomiting
- Coma

SURE PREVENTION CAN BE ADOPTED BY FOLLOWING MEASURES:

- Avoid water activities in pools / ponds / water etc or untreated
- Water when the water is warm or the temperature is near or over 80o F.
- Keep your head above water or use a NOSE CLIP.
- While nasal rinses use distilled, sterile, or previously boiled and cooled water.
- Periodical cleaning of water tanks and keeping it well chlorinated.

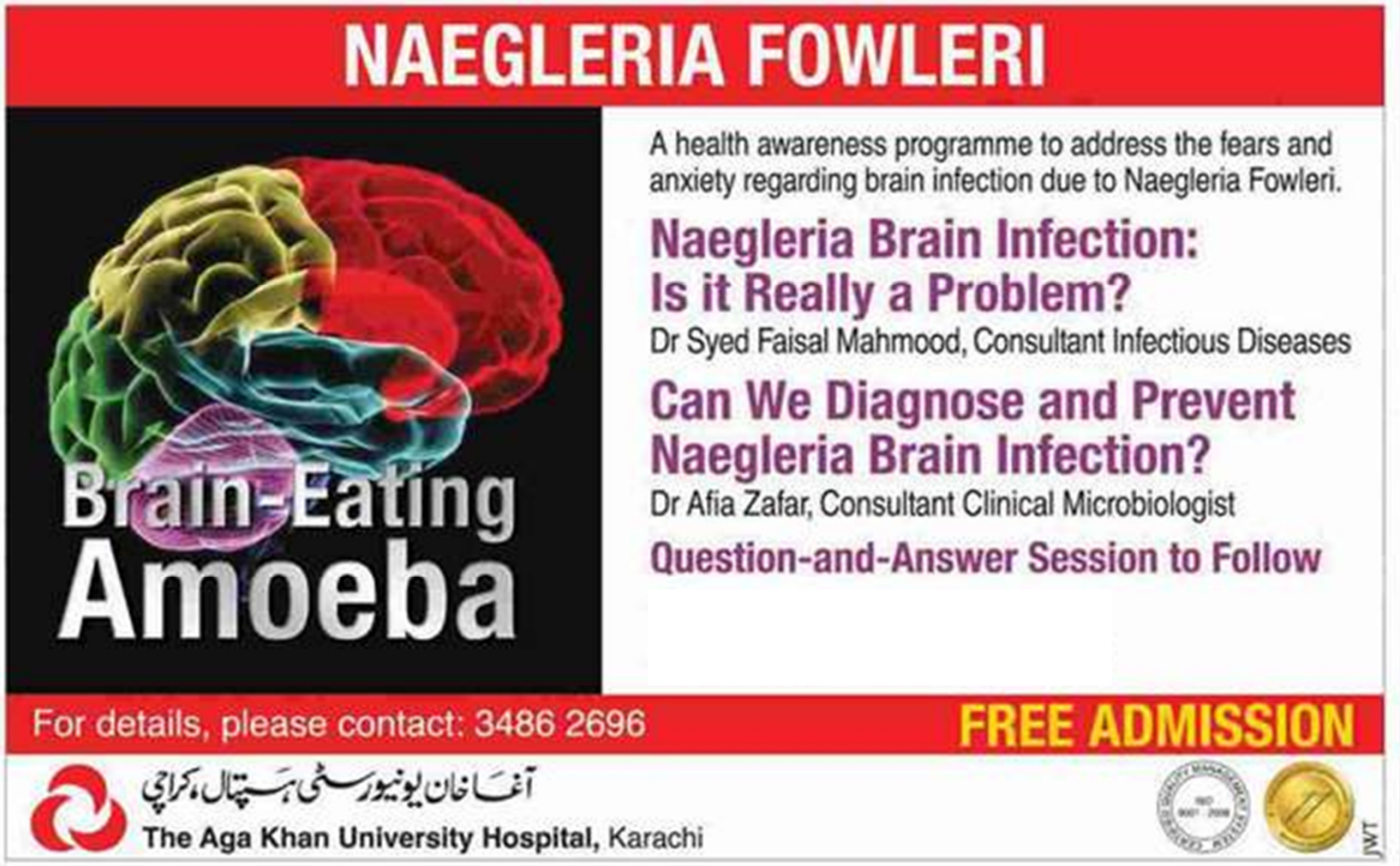
BUT THERE IS SURE PREVENTION

- One CANNOT get infected from drinking water contaminated with NAEGLERIA.
- One ONLY gets infected when contaminated water goes up into your nose.
- Naegleria is 99.9% FATAL having NO CURE
- It is 100% PREVENTABLE

INF/KRY NO. 1966/2014

Kill Naegleria Fowleri Before It Kills You

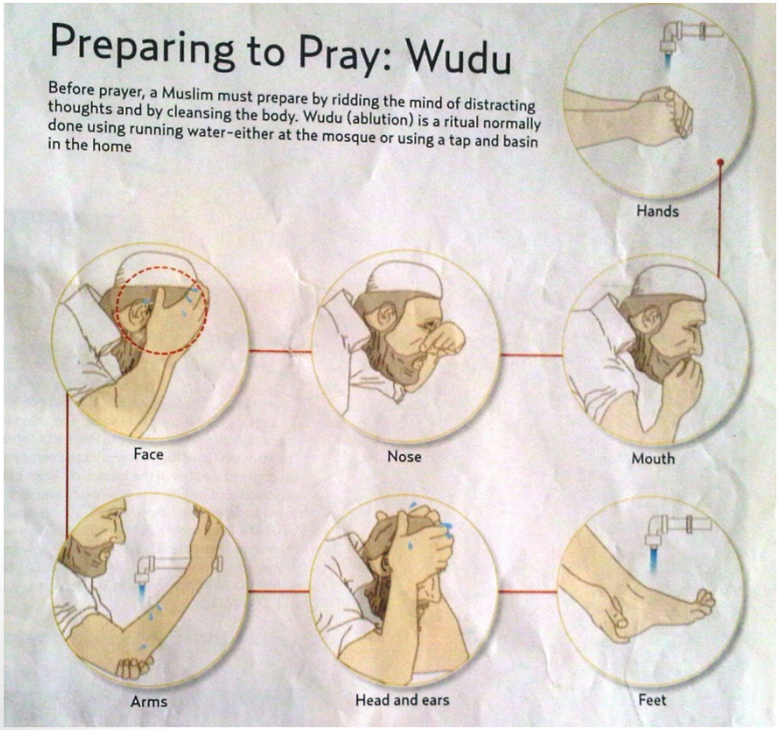
2014



44 cases only in Karachi!

Ablution




Preparing to Pray: Wudu
Before prayer, a Muslim must prepare by ridding the mind of distracting thoughts and by cleansing the body. Wudu (ablution) is a ritual normally done using running water–either at the mosque or using a tap and basin in the home
Hands
Face
Nose
Mouth
Arms
Head and ears
Feet



Safe Ritual Nasal Rinsing

Use safe water to protect yourself from a deadly brain infection.

Many tiny germs live in warm water. *Naegleria fowleri* is a water-loving ameba (very small germ) that is often found around the world in ponds, lakes, and rivers. It also can hide in pipes connected to tap water.

Naegleria is so small, even water that looks, smells, and tastes clean can have the amebae swimming in it. Nasal rinsing with unsafe water can lead to infection.



Many people perform ritual nasal rinsing. This practice is included in Yogic, Ayurvedic, and Islamic traditions.

Within the Islamic faith, ritual nasal rinsing is included in a cleansing process called "wudu" or "ablution." It is usually performed several times a day in preparation for prayer.

Use safe water to protect yourself.*

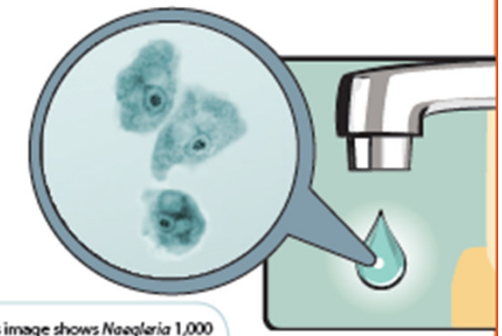
Take at least one of these actions to lower your risk of becoming infected with *Naegleria* during ritual nasal rinsing:

- **Boil:** Use water that has been previously boiled for 1 minute and left to cool.
  - At elevations above 6,500 feet, boil for 3 minutes.
- **Filter:** Use a filter designed to remove some water-loving germs.
  - The label may read "NSF 53" or "NSF 58."
  - Filter labels that read "absolute pore size of 1 micron or smaller" are also effective.
- **Buy:** Use water with a label specifying that it contains distilled or sterile water.
- **Disinfect:** Learn how to disinfect your water to ensure it is safe from *Naegleria*.
  - Chlorine bleach used at the right level and time will work as a disinfectant against this germ.

Infections from *Naegleria* are deadly and rare.

When water tainted with *Naegleria* is sniffed up the nose, the ameba can travel to the brain. This causes the disease **primary amebic meningoencephalitis (PAM)**, which destroys brain tissue and usually results in death.

- **Infections are deadly:** Of 132 people known to be infected in the United States from 1962 – 2013, only three people have survived.
  - Most *Naegleria* infections are due to swimming in warm lakes or rivers.
- **Infections are rare:** There has only been one reported infection associated with ritual nasal rinsing in the U.S.
  - More infections linked to ritual nasal rinsing have been reported globally.
  - Two infections have been linked to using neti pots or similar devices to rinse sinuses with salt solutions made from contaminated tap water.



This image shows *Naegleria* 1,000 times bigger than it really is.

Talk to your doctor if you have concerns.

Naegleria infection may be mild at first and include

PREVENTION


LA NARIZ
WARNING
DO NOT ALLOW WATER TO ENTER YOUR NOSE
NAEGLERIA FOWLERI
AN AMOEBA COMMON TO THERMAL POOLS MAY ENTER CAUSING A RARE INFECTION AND DEATH


WARNING
Swimming is not recommended in these waters
A thermophilic amoeba, Naegleria fowleri, has been found in the waters at Huckleberry / Polecat Creek.
This amoeba lives in naturally occurring warm waters and enters the body by ingestion. It is known to cause serious skin rashes, infections, and/or amoebic meningoencephalitis that can be fatal.


GEOTHERMAL POOL
KEEP YOUR HEAD ABOVE WATER
PREVENT AMOEBIC MENINGITIS
No jumping, diving, splashing or ducking

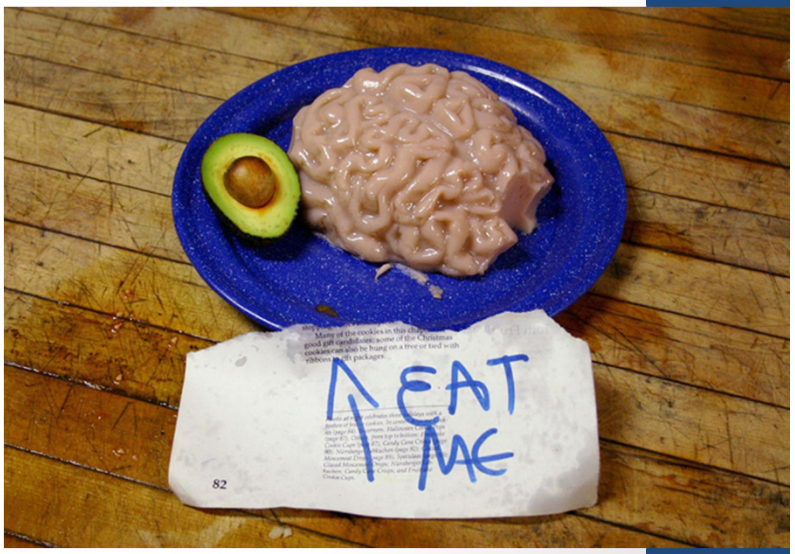
Amoeba Awareness



Prevention?


Brain-Eating Amoeba
GIANT microbes


I'LL EAT YOUR BRAIN
CAN'T, I DON'T HAVE ONE
NewsBlaze.com


EAT ME

PREVENTION AND CONTROL

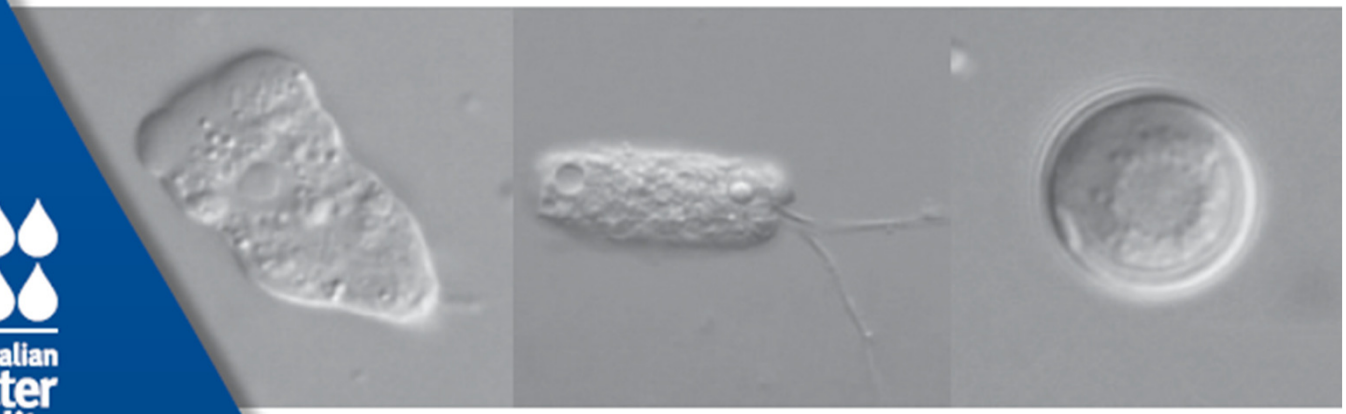
Government of Western Australia
Department of Health
Public Health and Clinical Services

Amoeba response protocol

Safe drinking water which has the trust of consumers

Australia cares about amoebae

Pathogenic Free-Living Amoebae



Australian Water Quality Centre

Analysis for:
Naegleria
Acanthamoeba

Limit of Reporting:
<1/L

There are several groups of amoebae that are natural aquatic or soil organisms but are also opportunistic pathogens; that is, infection is coincidental to their normal life cycle.

Naegleria Fowleri

The waterborne disease primary amoebic

NO REPORTED CASES IN 20 YEARS

Costa Rica- USA: july/august 2014



Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People.™



San Carlos (CR)
Turism
Politics

LA NACIÓN

NACIO

(/NACIO

my

ORLAND

Ford of CLERMONT
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OUR PRICE IS JUST PLA
LOWER EVERYDA
HUGE SELECTION! LOWEST PRI

Sanford family s deadly amoeba

Posted: Aug 15, 2014 12:42 PM
Updated: Aug 22, 2014 12:42 PM

By Ryan Elijah, Anchor

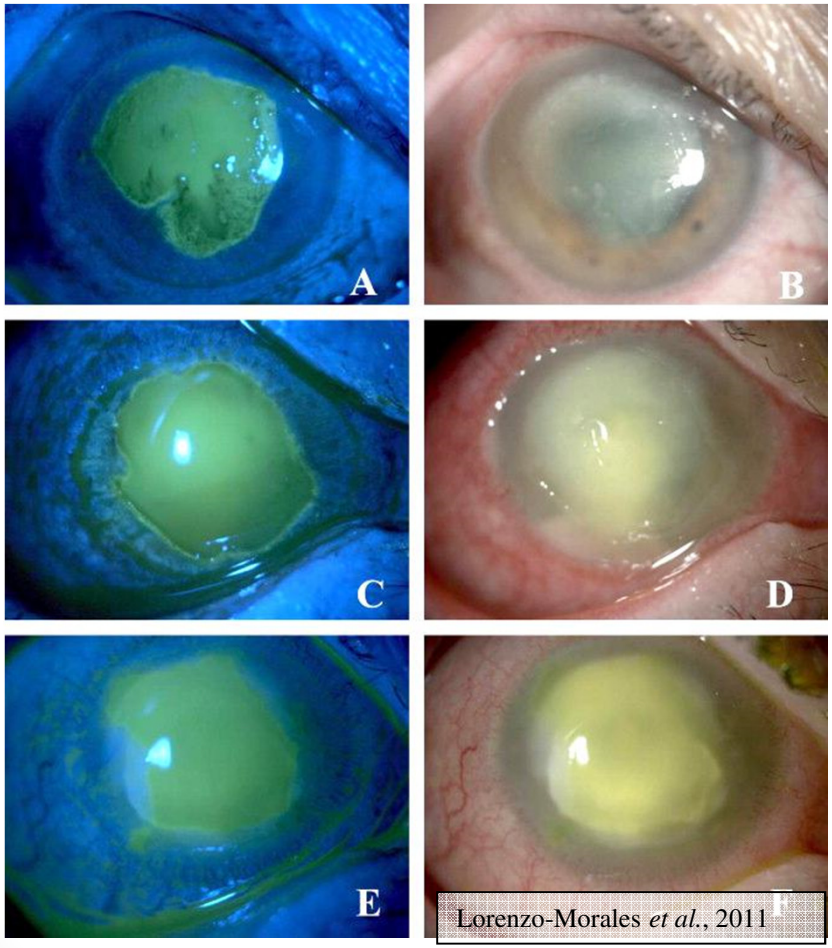
SANFORD, Fla. (WOFL FOX 35 ORLANDO) - A dream vacation for one local family recently ended with the tragic death of their only child.
They're sharing their nightmare for the first time, with the hope that no family has to ever experience what they've been through.

11-year-old Jordan Smelski died in July after being infected with a rare amoeba found in freshwater. He was the third person to die from the rare infection in a three-week span. Living in Florida, the family never allowed their son to swim in lakes or rivers due to the risk of the amoeba, but they didn't realize it could also live in hot springs.

"it brought us to our knees, we were educated, but we didn't have all the information", said Shelly Smelski, Jordan's Mother.

Steve Smelski and his son swam for hours in a hot springs at a resort in Costa Rica, a place the deadly amoeba Naegleria Fowleri could exist.

Acanthamoeba spp.



Lorenzo-Morales *et al.*, 2011

- Invasive infection of the cornea.
- Non specific symptons.
- Incidence rising.

Main risk group:
Contact Lens Wearers

Acanthamoeba spp. Therapy

Current first line treatments:

- Topical application of **diamidines** (propamidine isotionate or hexamide)
- And cationic antiseptics: **biguanides** (polihexametilenbiguanide , (PHMB) or chlorhexidine digluconate)





WHAT WE DO IN OUR LAB

- Therapy
  - Chemotherapy→ novel active principles and products in the market
  - Molecular Therapy→ siRNA
  - Combination→ elucidation of drug targets
- Translational Medicine

ISSUES TO SOLVE

- FLA biology is still under study
- Axenic media are required for *B. mandrillaris* chemotherapy assays
- Pathogenecity is poorly understood.
- Cyst stage
- In vitro FLA chemotherapy assays → standardization
- *In vivo* models

TARGETS

Cytoskeleton (Actin, myosin, α & β tubulin)
Inhibitors: unknown (current known inhibitors have no effect)

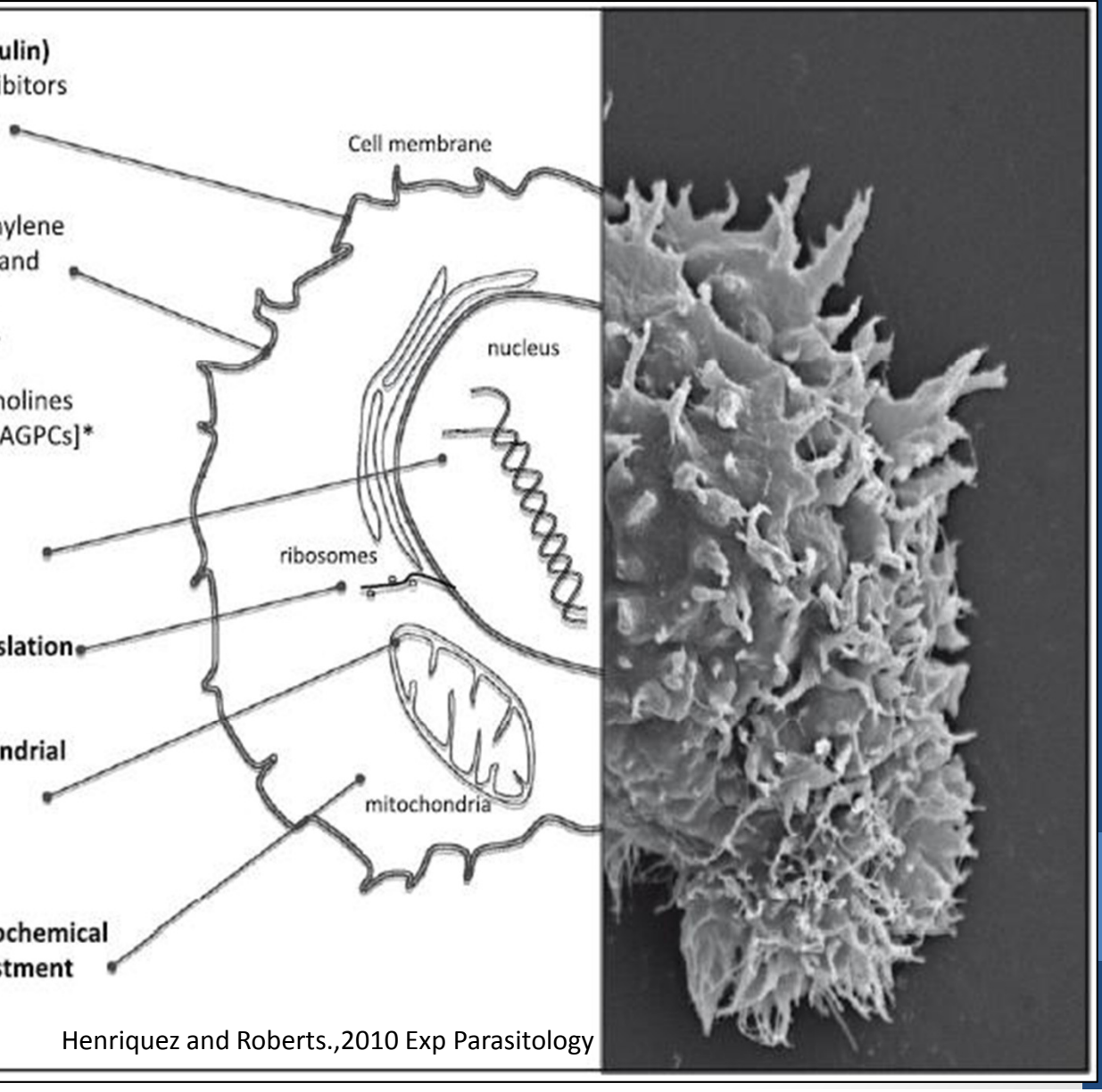
Membrane
Inhibitors: chlorhexidene, polyhexamethylene biguanide, acriflavine hydrodrochloride and proflavin, polymyxin B, amphotericin B, ketoconazole, miconazole, clotrimazole, fluconazole target sterols.
Phospholipid analogues (alkylphosphocholines [APCs] & alkylglycerolphosphocholines [AGPCs]*

Nucleic Acid
Inhibitors: 5-fluorocytosine (5-FC), RNAi

Polyamines biosynthesis (ornithine decarboxylase), effects on protein translation
Inhibitors: DFMO

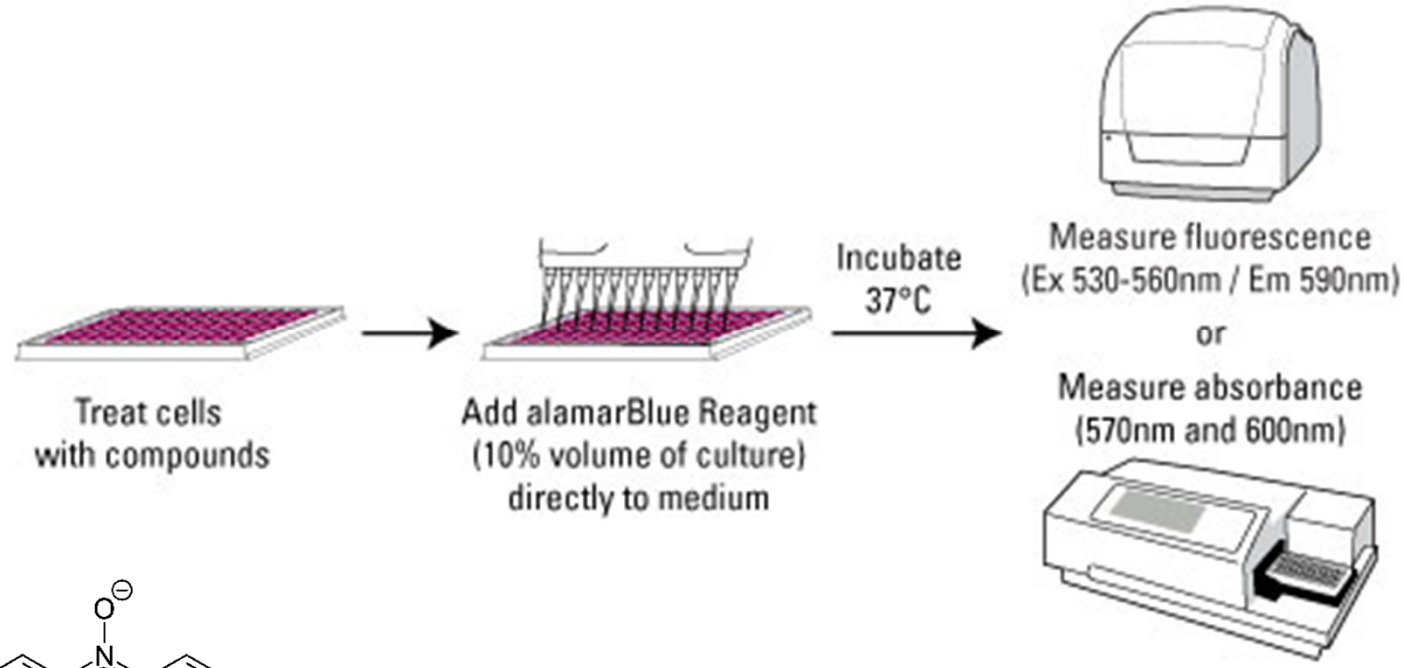
Alternative oxidase (AOX) and mitochondrial respiration
Inhibitors: SHAM, Ascofuronone,

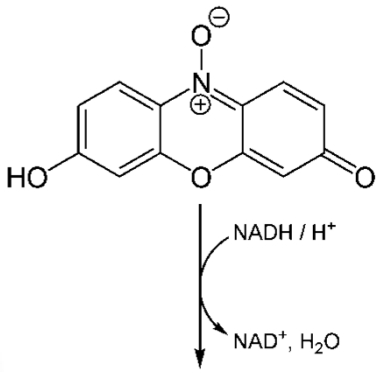
Differentiation to cysts
Cellulose Biosynthesis and Proximal Biochemical (e.g. glycogen metabolism) affect encystment
Inhibitors: 2,6-bichlorobenzonitrile



Henriquez and Roberts.,2010 Exp Parasitology

Alamar Blue Assay



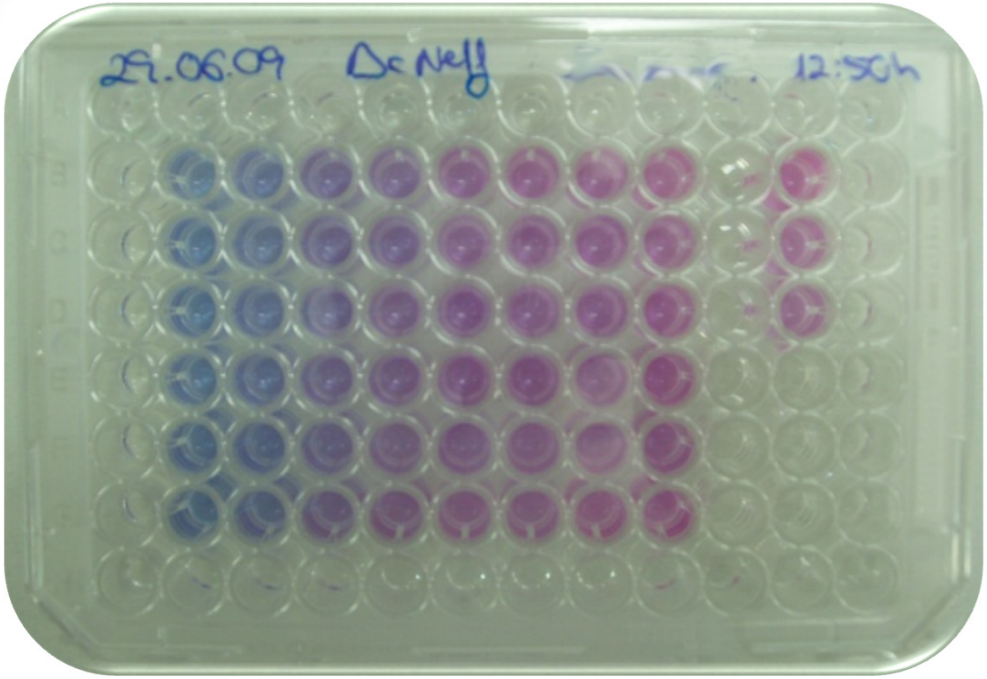


Resazurin blue

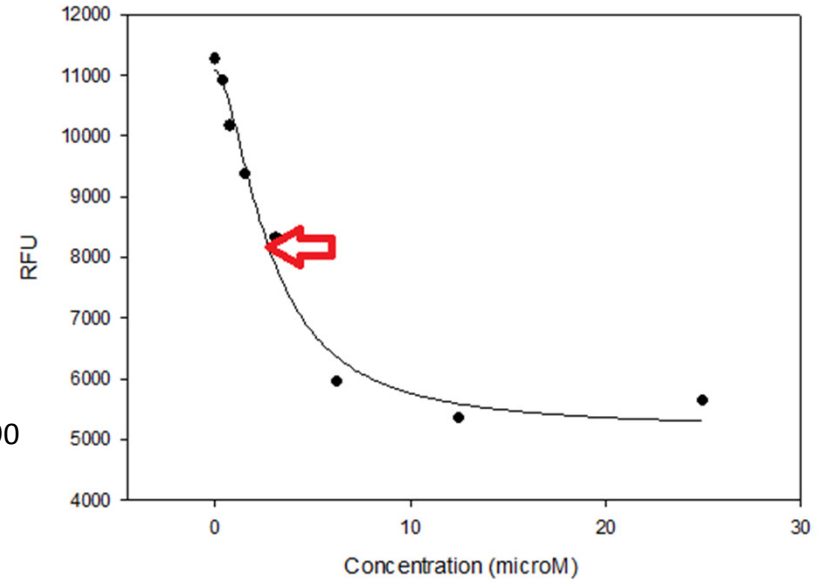
Resorufin pink



Chemotherapy assays

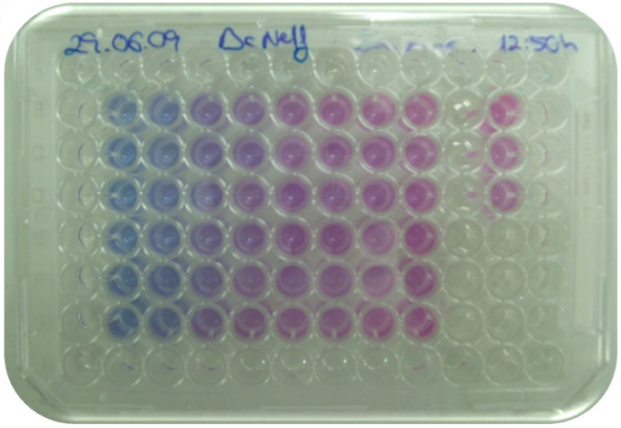


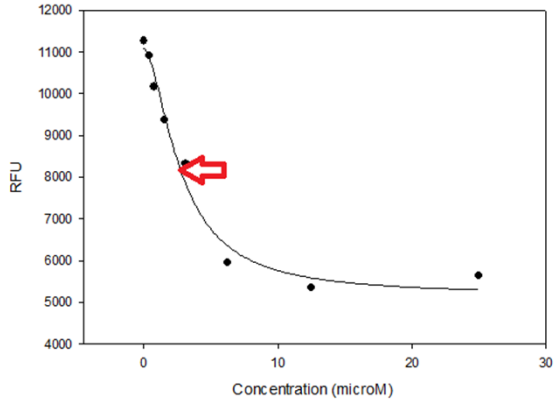
IC_{50} and IC_{90}

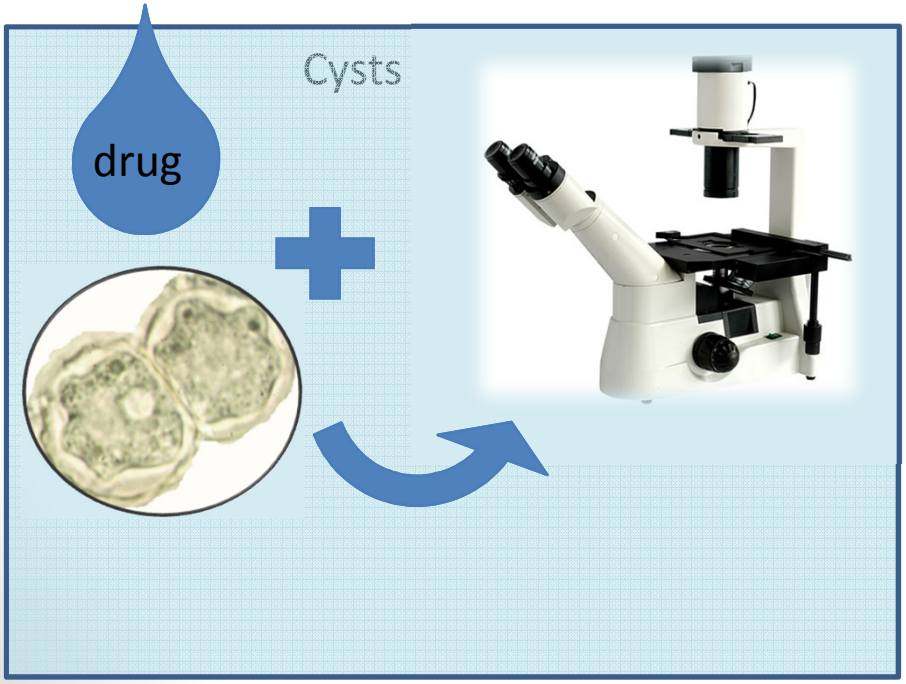


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Chemotherapy assays



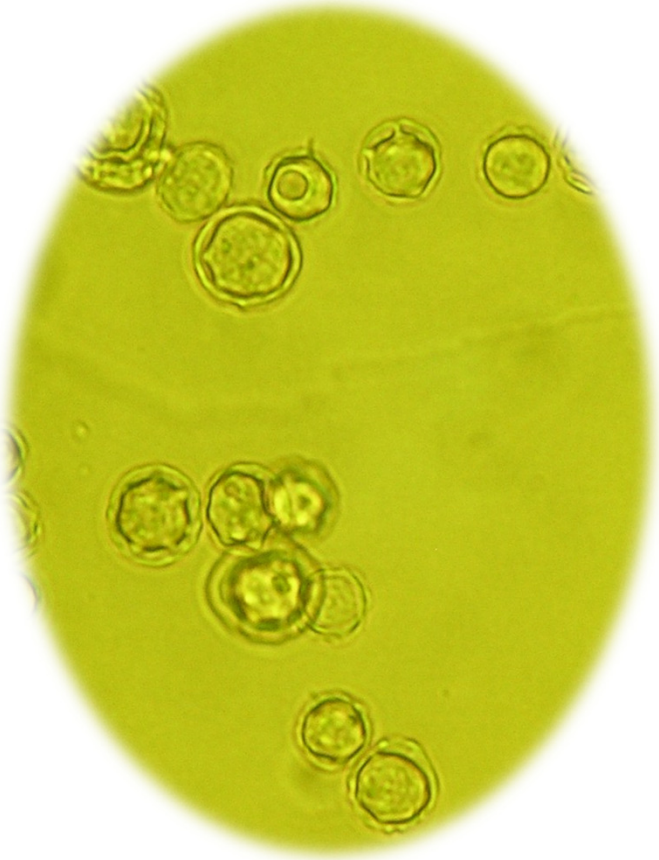


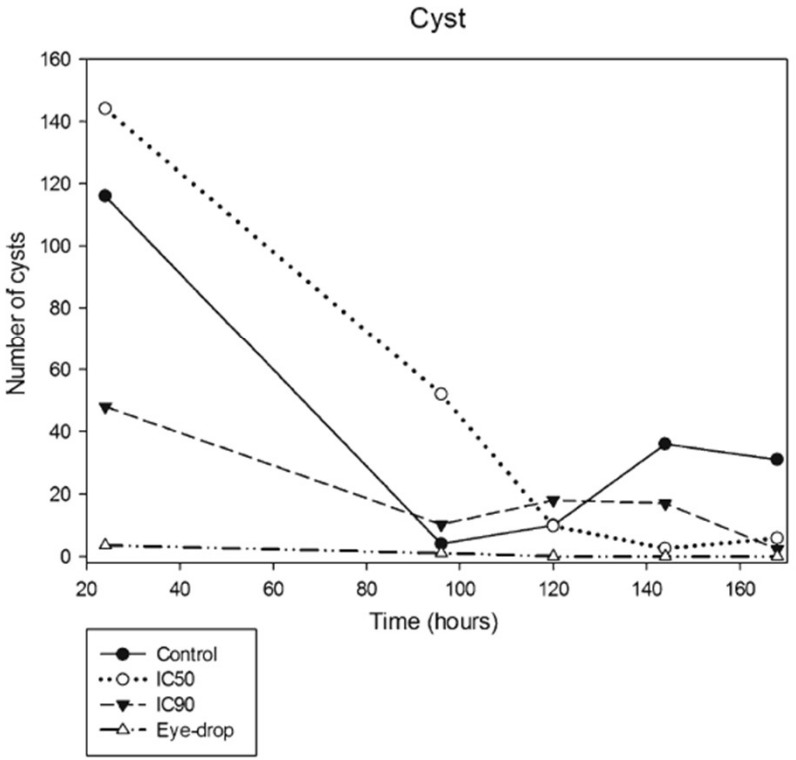




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Chemotherapy assays





Cysticidal activity

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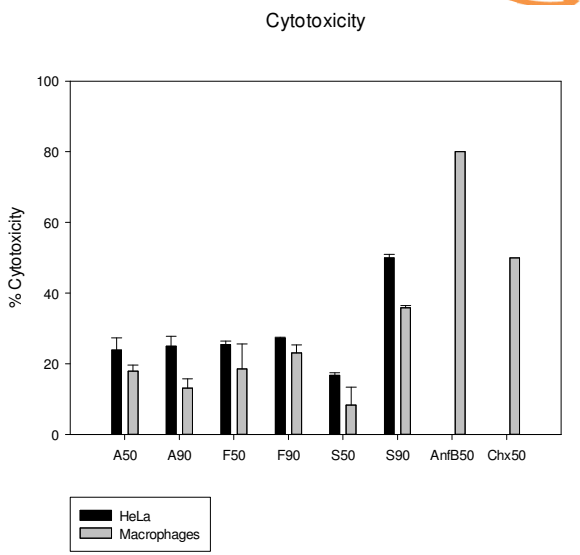
Cytotoxicity Evaluation

Cytotoxicity Detection Kit (LDH)®
Roche → LDH activity

IC_{50} and IC_{90}
Tested compounds

Macrophages J774.1A
HeLa





0-10 %: Non toxic
10-25 %: Low
25- 40 %: Moderated
>40 %: High

(Lorenzo-Morales *et al.*, 2010)

Our sources

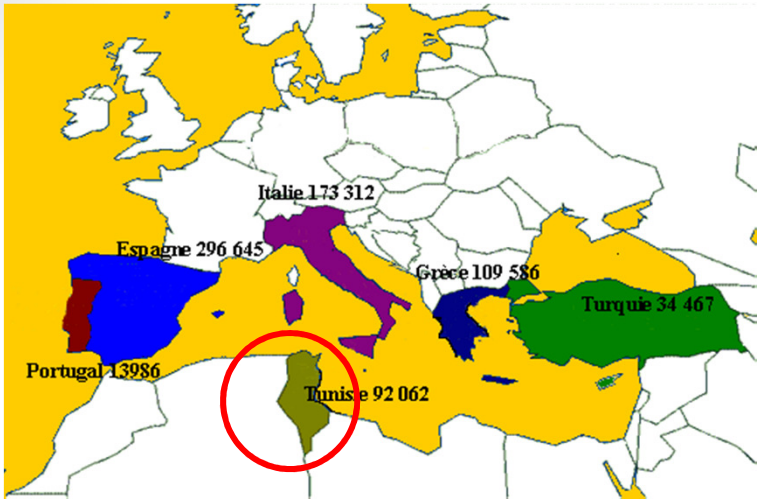
- Natural origin→ Plants (medicinal, folk/ traditional…)
- Synthetic
- Comercialized products→ eye drops, …











Tunisia

Olive tree leaves,
Used in traditional medicine

Zarrazi, Limouni, Chemlali Tataouine, Dhokkar, and Toffehi



Dhokkar



Limouni

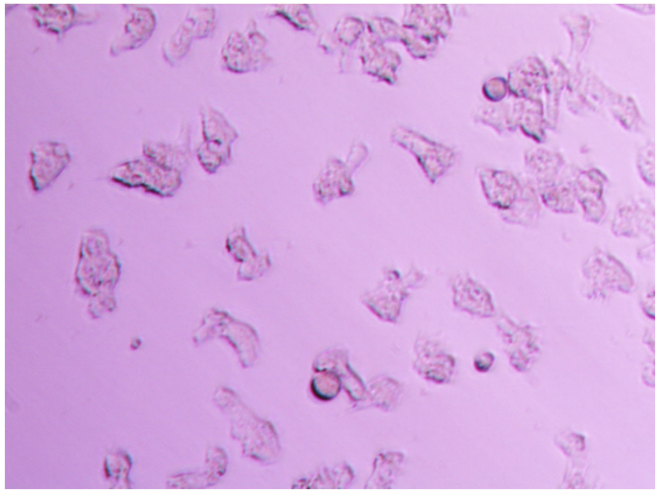


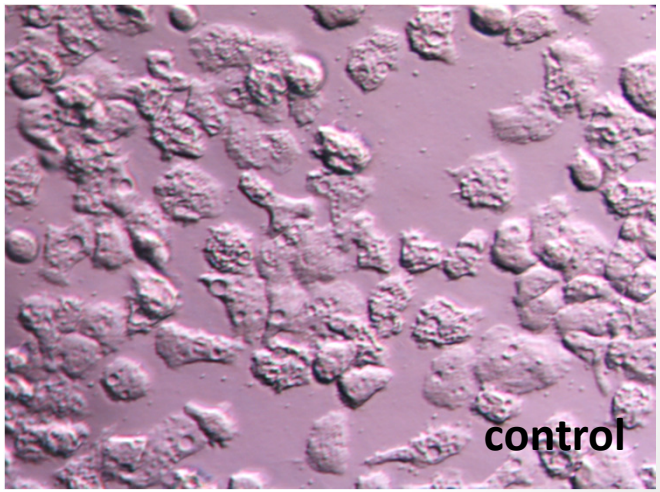
Chemlali Tataouine

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Total extracts of OLE against *A. castellanii* Neff

Cultivar		Limouni	Zarrazi	Dhokkar	Toffehi	Chemlali Tataouine
IC_{50} (µg/ml)	Methanol	12.585 ± 1.514	23.828 ± 0.732	10.108 ± 2.953	33.661 ± 1.398	22.083 ± 1.379
	Ethanol	17.505 ± 3.198 bcB	26.172 ± 1.367	10.126 ± 0.189	22.992 ± 1.703	19.495 ± 0.378
	Mixture	9.369 ± 1.325	19.397 ± 2.271	8.234 ± 1.703	24.402 ± 2.356 cB	19.617 ± 0.183





Parasitology Research
August 2013, Volume 112, Issue 8, pp 2825-2829
Activity assessment of Tunisian olive leaf extracts against the trophozoite stage of *Acanthamoeba*

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Dhokkar crude extract

Methanol fraction

Ethyl-acetate fraction

Chromatography

ARTICLE IN PRESS

Experimental Parasitology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr



L

Bioassay guided isolation and identification of anti-*Acanthamoeba* compounds from Tunisian olive leaf extracts

Ines Sifaoui [a,*], Atteneri López-Arencibia [b,2], Juan Carlos Ticona [c], Carmen Mª Martín-Navarro [b,d,2], María Reyes-Batlle [b,2], Mondher Mejri [a,1], Jacob Lorenzo-Morales [b,2], Antonio Ignacio Jiménez [c], Basilio Valladares [b,2], Isabel Lopez-Bazzocchi [c], Manef Abderabba [a,1], José E. Piñero [b,2]

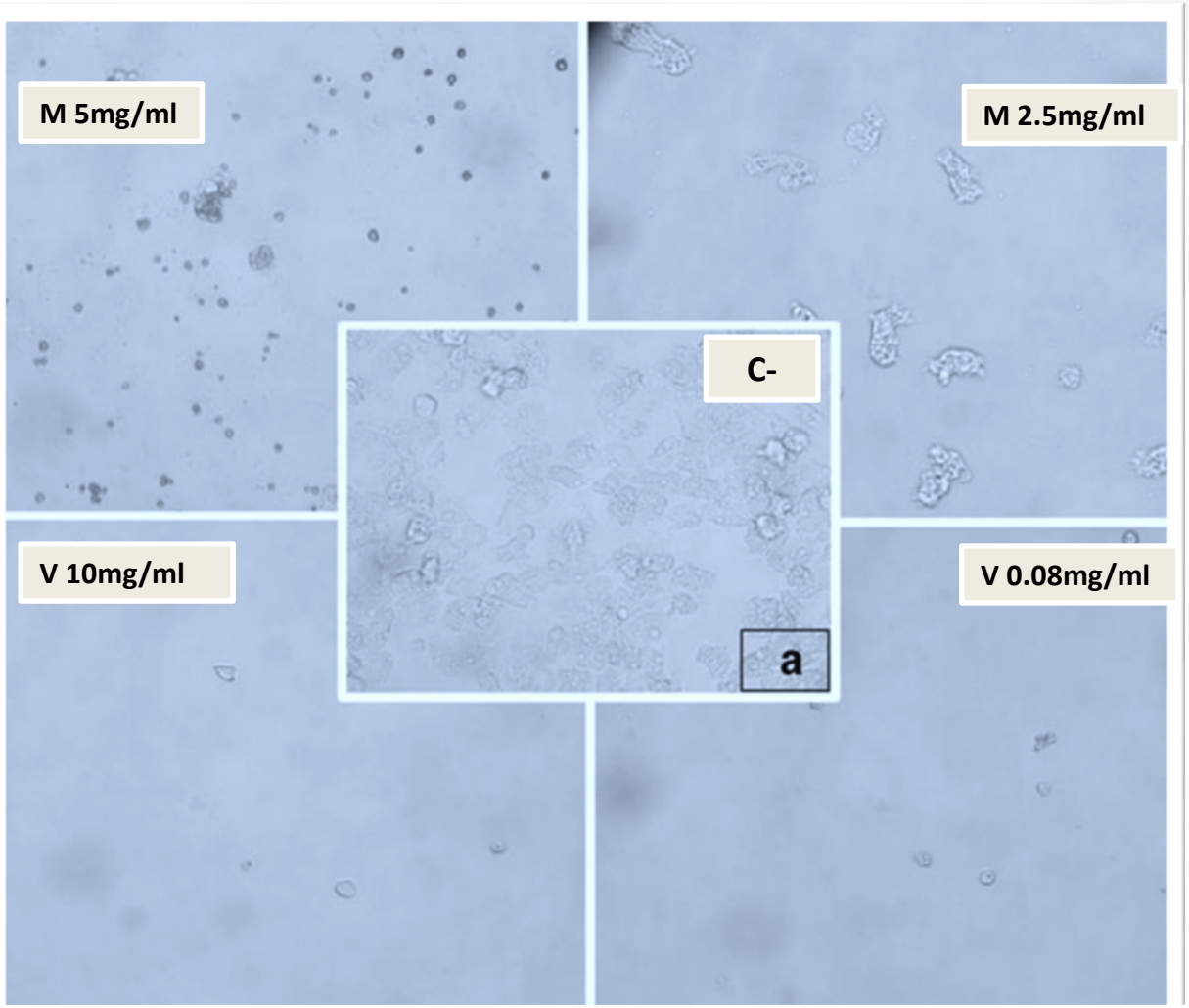
[a] *Laboratoire Matériaux-Molécules et Applications, IPEST, B.P. 51, 2070 La Marsa, University of Carthage, Tunisia*
[b] *University Institute of Tropical Diseases and Public Health, University of La Laguna, Avda Francisco Sanchez s/n, Campus de Anchieta, 38271 la Laguna, Tenerife, Canary Islands, Spain*
[c] *Instituto Universitario de Bio-Orgánica Antonio González, Universidad de La Laguna, Tenerife, Canary Islands, Spain*
[d] *Centre for Integrative Physiology, School of Biomedical Sciences, University of Edinburgh, Edinburgh, Scotland, United Kingdom*





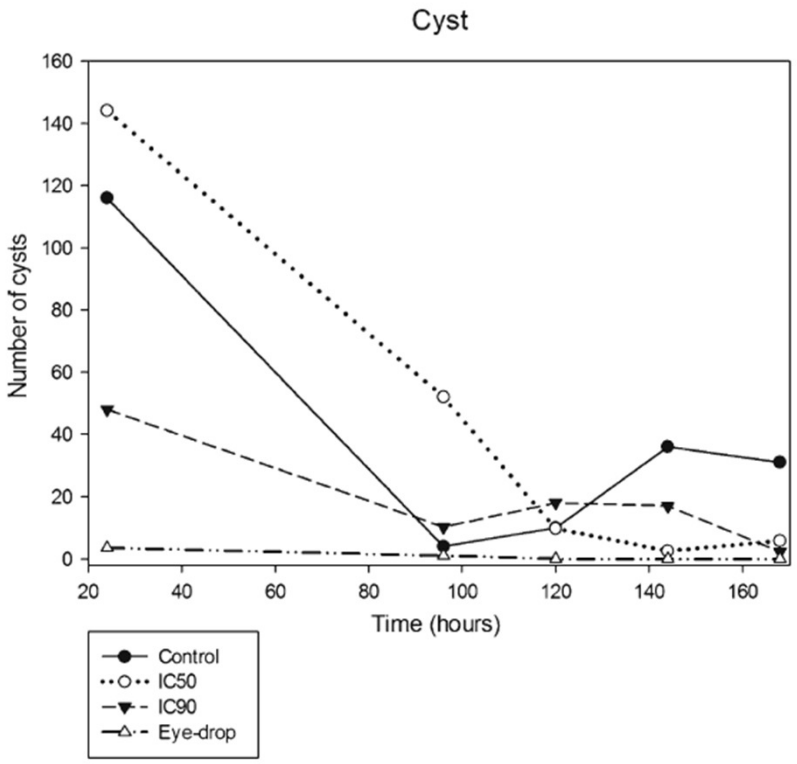
- **Moxifloxacin** eye drops solution (VIGAMOX®) Alcon (0.5 %, 5 mg/ml)
- **Voriconazole** (VFEND®) by Roerig/Pfizer Inc. (1%, 10 mg/ml)
- Clorhexidine digluconate , Alfa Aesar (0.1 %, 1 mg/ml)
- Anfotericine B**,** Sigma-Aldrich Chemistry Ltd (0.1 %, 1 mg/ml)

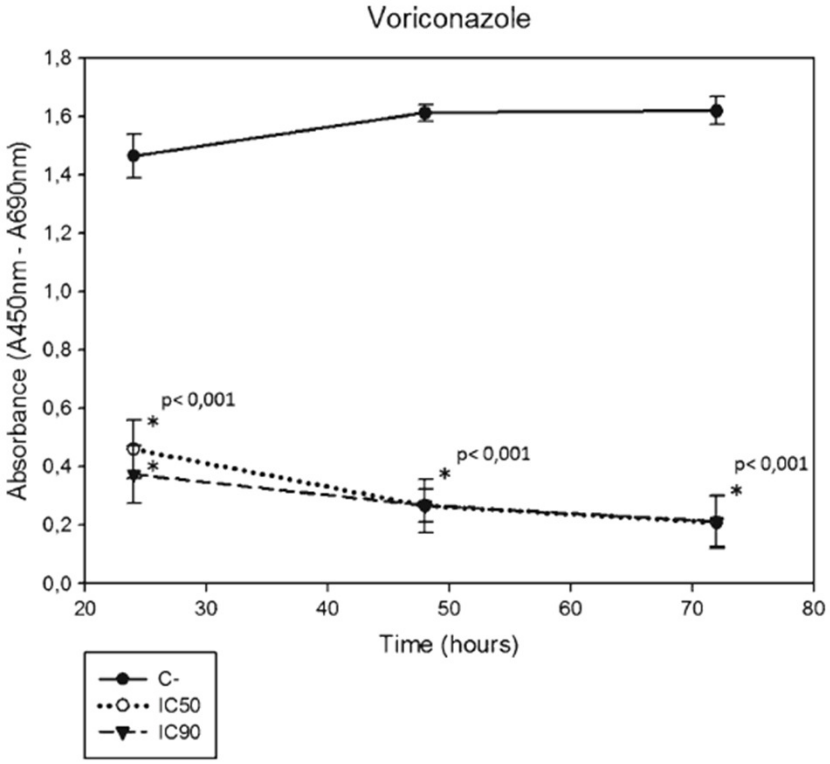
- Type strain: *Acanthamoeba castellanii* Neff (ATCC 30010, genotype T4)
- Clinical Strains (CLC-16, genotype T3 and CLC-51, genotype T1)



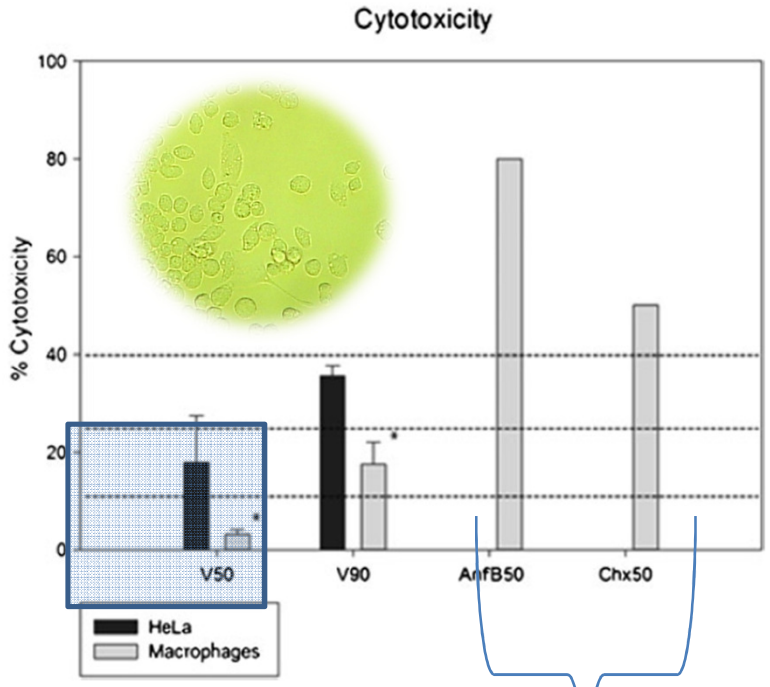
(µg/ml) 96h	AcNeff		CLC-16		CLC-51.1	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Voriconazole	4.59±0.24	10.63±0.46	7.66±2.05	15.43±4.00	4.59±0.59	10.48±1.37
Moxifloxacin	2,630.00±400.00	3,920.00±10.00	180.00±120.00	680.00±50.00	879.93±110.00	150.00±130.52
Chlorhexidine	0.92±0.10	8.16±0.45	6.01±1.92	17.42±1.23	5.29±0.75	14.26±0.91
Amphotericin B	36.61±0.52	92.36±8.79	22.82±1.18	66.39±3.14	62.15±5.24	157.09±12.67

Voriconazole





Cytotoxicity Evaluation



Graefes Arch Clin Exp Ophthalmol
DOI 10.1007/s00417-013-2371-y

BASIC SCIENCE

Evaluation of the *in vitro* activity of commercially available moxifloxacin and voriconazole eye-drops against clinical strains of *Acanthamoeba*

C. M. Martín-Navarro · A. López-Arencibia · F. Arnalich-Montiel · B. Valladares · J. E. Piñero · J. Lorenzo-Morales

Received: 10 January 2013 /Revised: 8 April 2013 /Accepted: 25 April 2013
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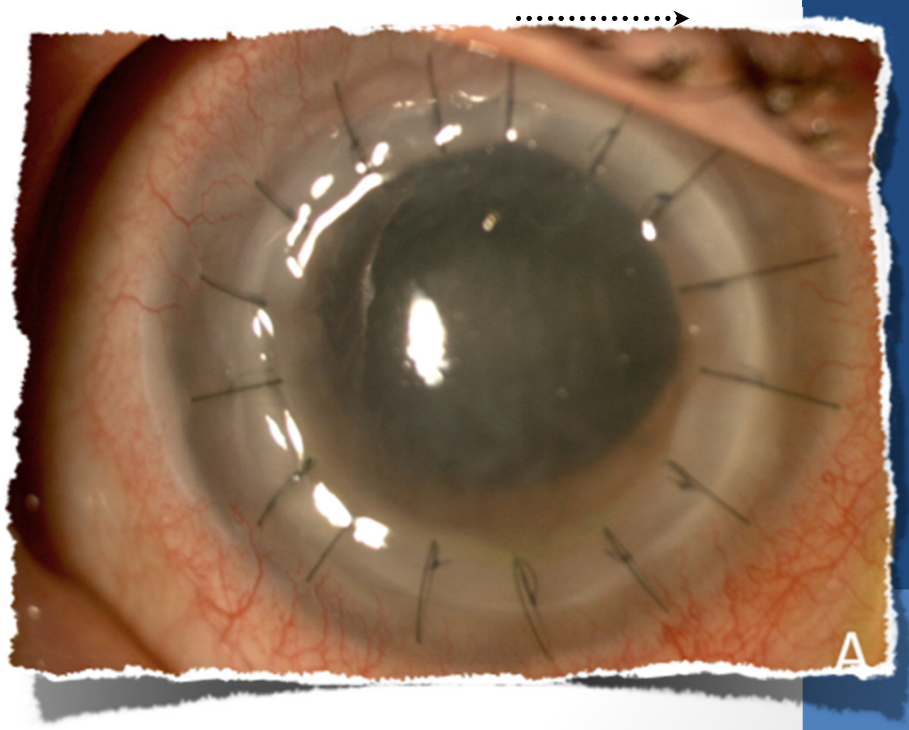
RESEARCH LETTERS

Successful Monitoring and Treatment of Intraocular Dissemination of *Acanthamoeba*

Francisco Arnalich-Montiel, MD, PhD
Carmen M. Martín-Navarro, PhD
Jorge L. Alió II, MD
Rogelio López-Vélez, MD, PhD
Enrique Martínez-Carretero, PhD
Basilio Valladares, PhD
José E. Piñero, PhD
Jacob Lorenzo-Morales, PhD

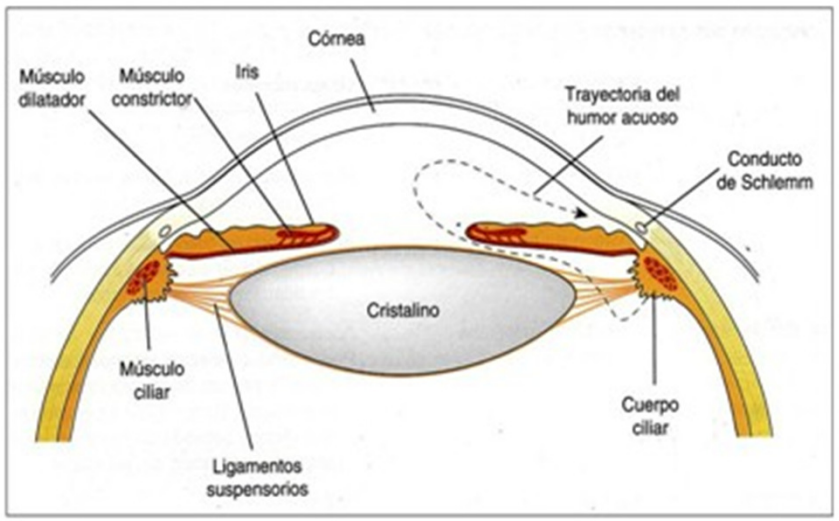
Successful customized voriconazole therapy for intraocular dissemination of *Acanthamoeba*.

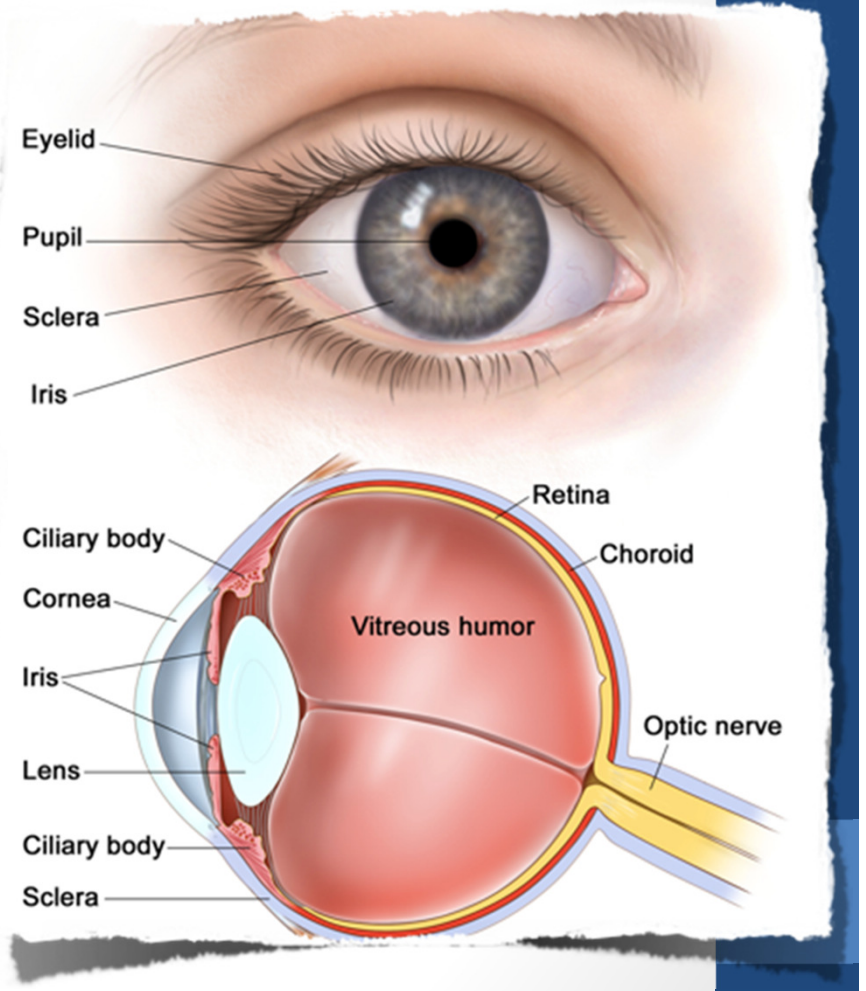
- AK → extracorneal, ocular perforation
- Keratoplastia
- Inflammation after 5 days



Successful customized voriconazole therapy for intraocular dissemination of *Acanthamoeba*. Case report and review of literature.

- Presence of *Acanthamoeba* Aqueous humor
- The clear fluid filling the space in the front of the eyeball between the lens and the cornea

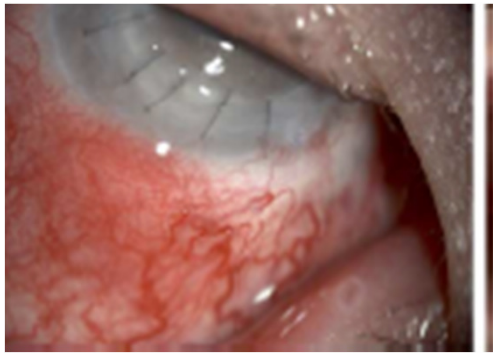


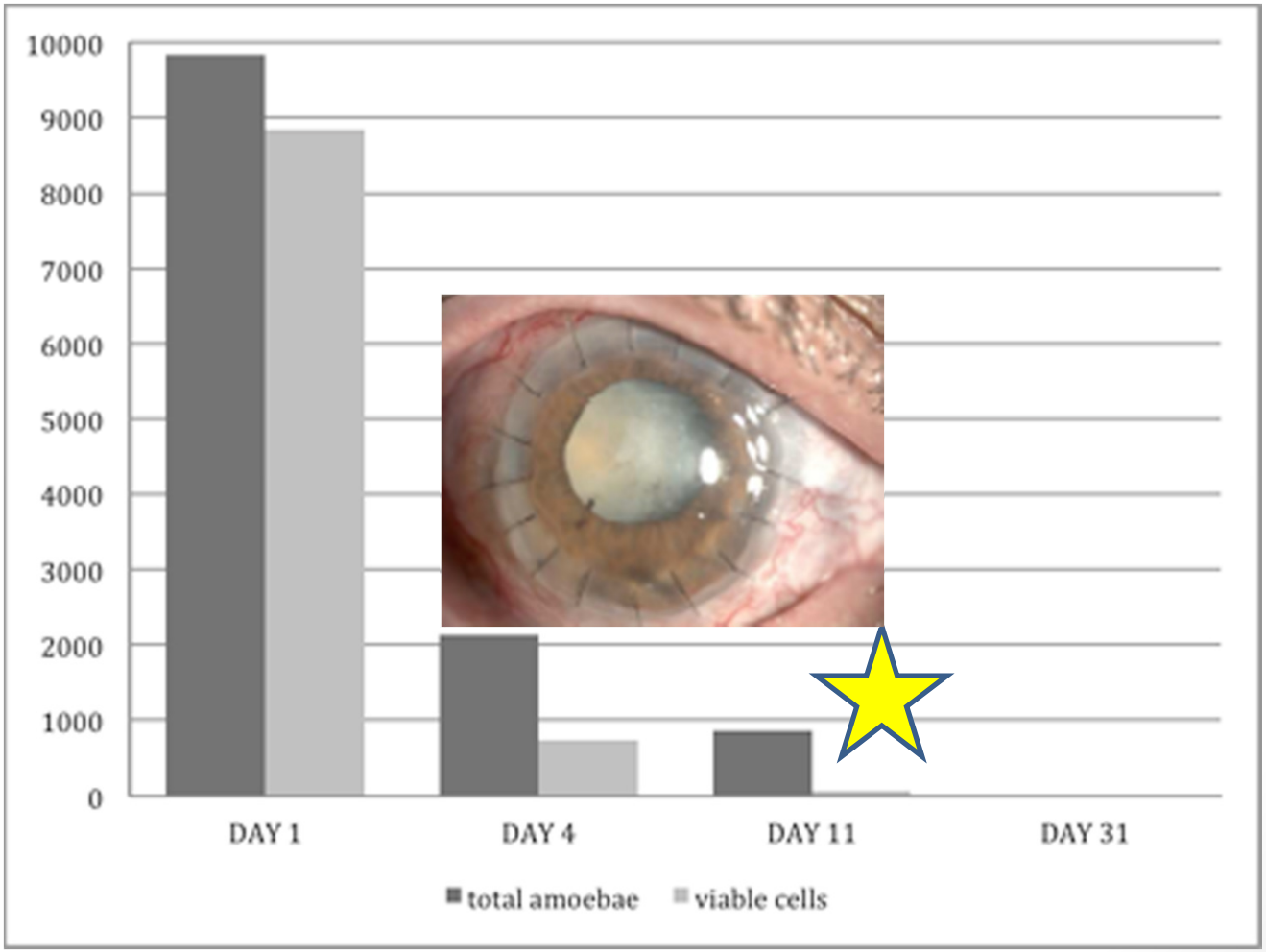


Successful customized voriconazole therapy for intraocular dissemination of *Acanthamoeba*. Case report and review of literature.

- T4 genotype Voriconazole sensitive
  - (IC90 10.63 µg/ml)
- Intraocular voriconazole 100 µg in serial 0.1 ml doses
- Oral voriconazol
- Chlorhexidine and topic voriconazol






10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
DAY 1
DAY 4
DAY 11
DAY 31
total amoebae
viable cells

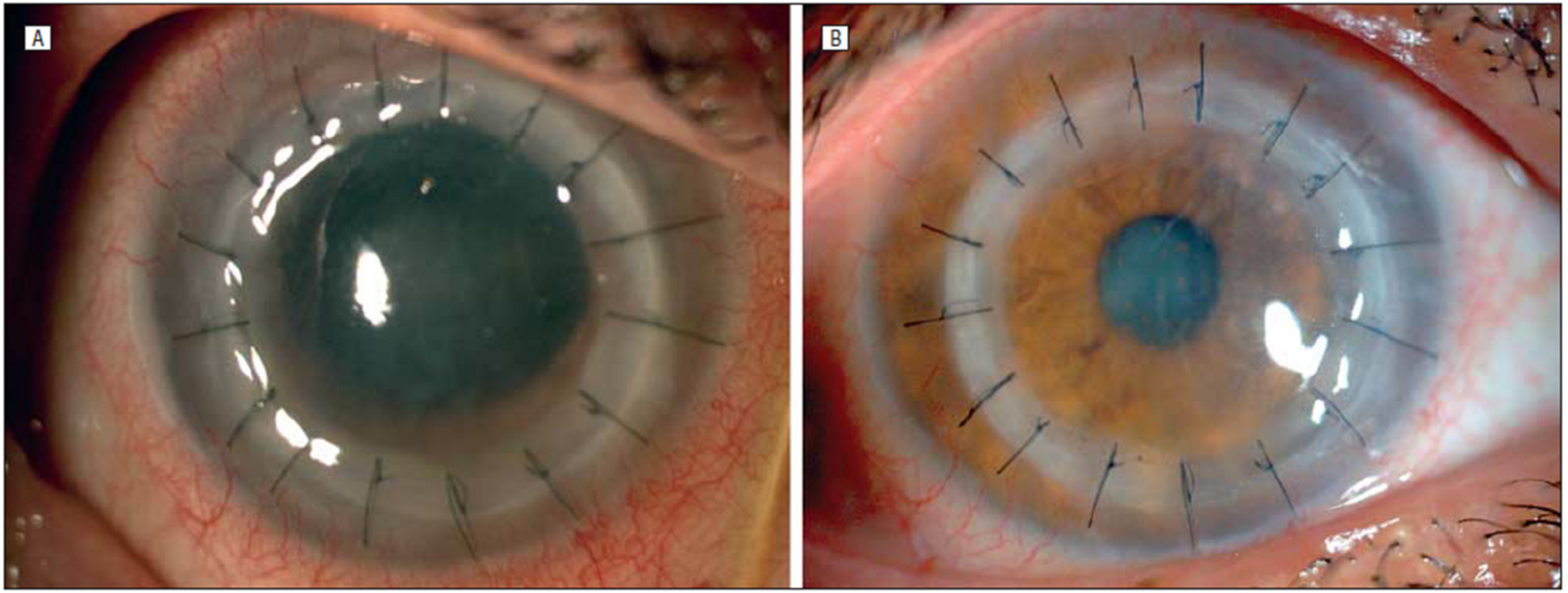
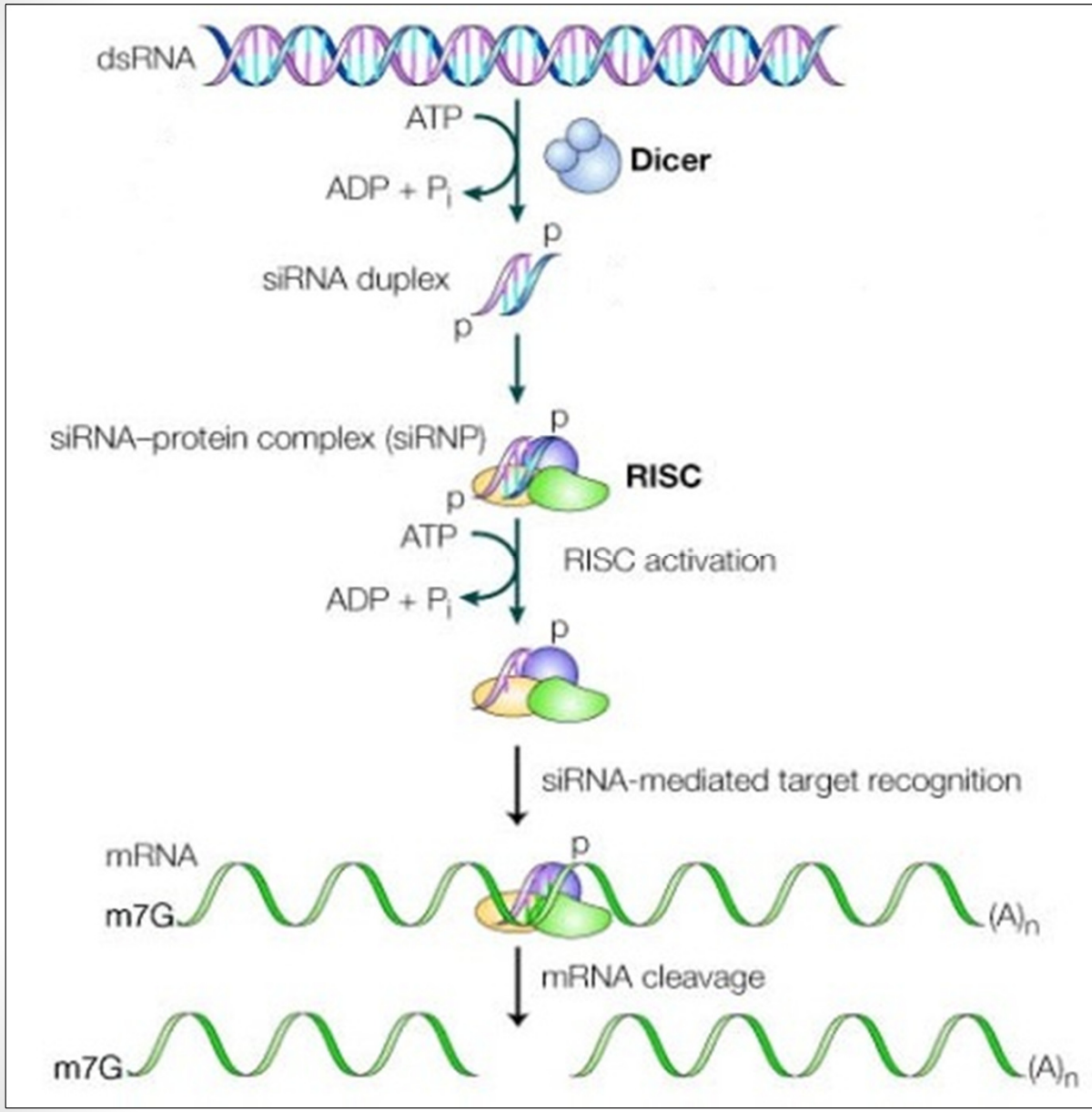
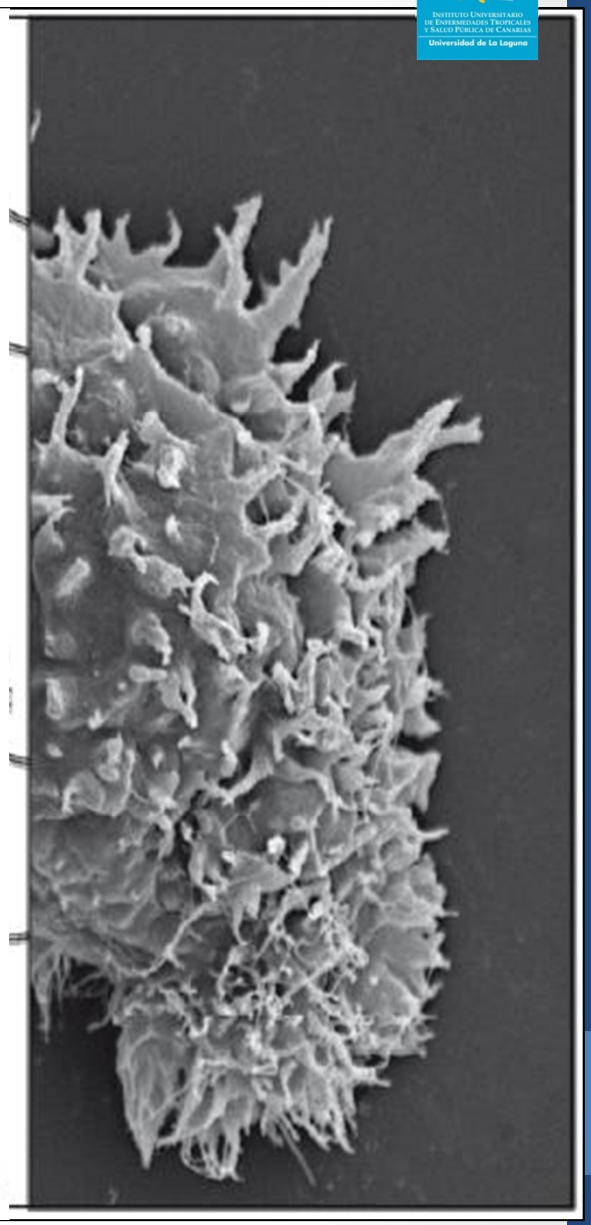


Figure 1. Anterior segment photograph of the patient's right eye 5 days after the therapeutic penetrating keratoplasty shows perikeratic hyperemia, corneal edema with Descemet folds, and mutton-fat keratic precipitates (A), and slitlamp examination after clinical resolution shows early signs of graft failure and pigmented keratic precipitates (B).

BEFORE

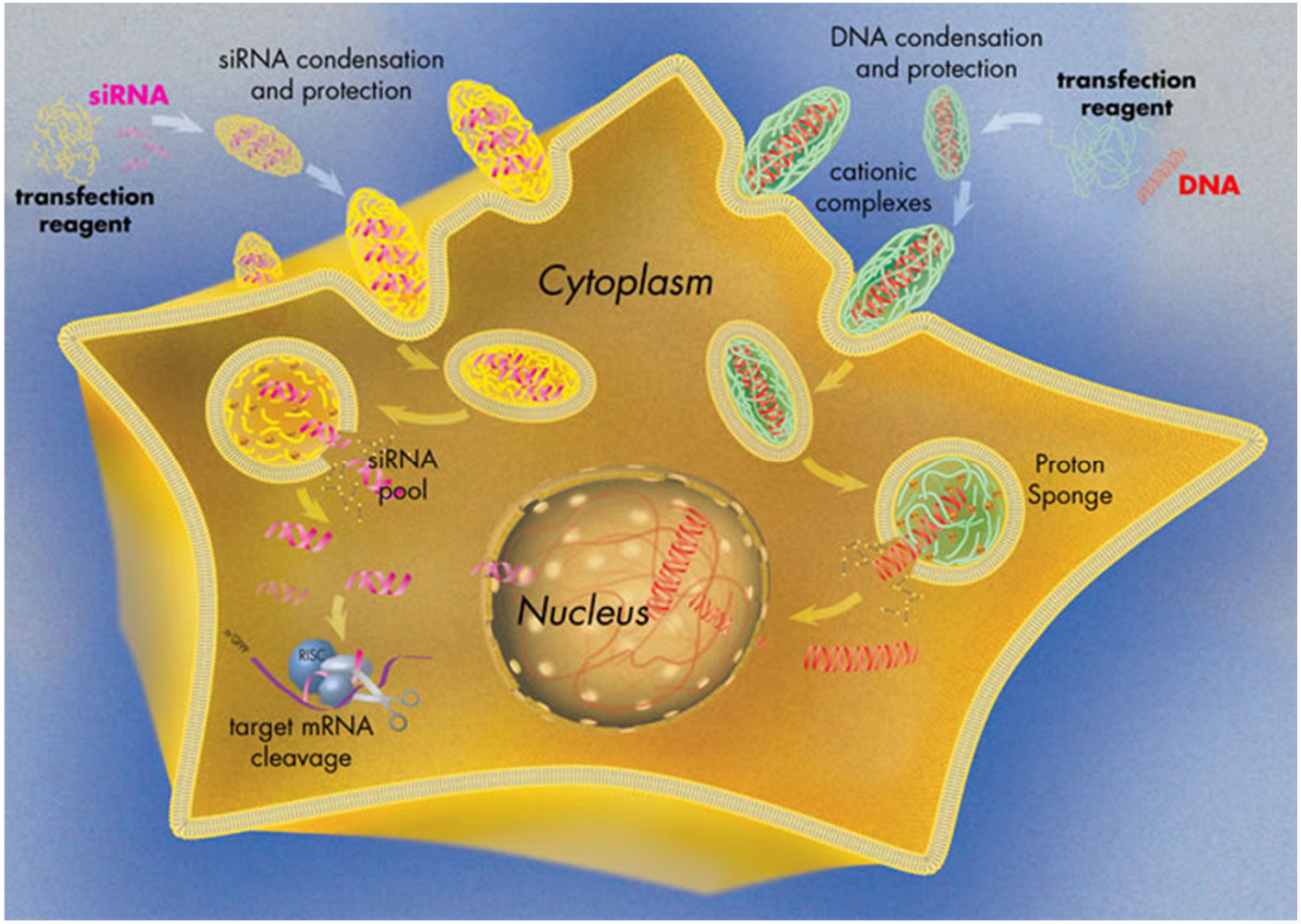
AFTER


dsRNA
ATP
ADP + Pi
Dicer
siRNA duplex
p
p
siRNA–protein complex (siRNP)
RISC
ATP
ADP + Pi
RISC activation
siRNA-mediated target recognition
mRNA
m7G
(A)n
mRNA cleavage
m7G
(A)n



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siRNAs



MOLECULAR THERAPY

- Extracellular serine proteases
  - Entry was blocked

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Molecular & Biochemical Parasitology 144 (2005) 10–15

MOLECULAR & BIOCHEMICAL PARASITOLOGY

RNA interference (RNAi) for the silencing of extracellular serine proteases genes in *Acanthamoeba*: Molecular analysis and effect on pathogenecity

Jacob Lorenzo-Morales, Antonio Ortega-Rivas, Pilar Foronda, Néstor Abreu-Acosta, David Ballart, Enrique Martínez, Basilio Valladares *

Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias, University of La Laguna, Avda. Astrofísico Fco. Sánchez, S/N 38203 La Laguna, Canary Islands, Spain

EUKARYOTIC CELL, Mar. 2008, p. 000 Vol. 7, No. 3
1535-9778/08/$08.00+0 doi:10.1128/EC.00316-07
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Glycogen Phosphorylase in *Acanthamoeba* spp.: Determining the Role of the Enzyme during the Encystment Process Using RNA Interference▽

Jacob Lorenzo-Morales,[1] Jarmila Kliescikova,[2] Enrique Martinez-Carretero,[1] Luis Miguel De Pablos,[3] Bronislava Profotova,[2] Eva Nohynkova,[2] Antonio Osuna,[3] and Basilio Valladares[1]*

University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, Tenerife, Canary Islands, Spain[1]; Department of Tropical Medicine, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic[2]; and Institute of Biotechnology, Department of Parasitology, University of Granada, Campus de Fuentenueva 18071, Granada, Spain[3]

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2010, p. 5151–5155 Vol. 54, No. 12
0066-4804/10/$12.00 doi:10.1128/AAC.00329-10
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Therapeutic Potential of a Combination of Two Gene-Specific Small Interfering RNAs against Clinical Strains of *Acanthamoeba*▽

Jacob Lorenzo-Morales,[1,2]* Carmen M. Martín-Navarro,[1] Atteneri López-Arencibia,[1] María A. Santana-Morales,[1] Raquel N. Afonso-Lehmann,[1] Sutherland K. Maciver,[2] Basilio Valladares,[1] and Enrique Martínez-Carretero[1]

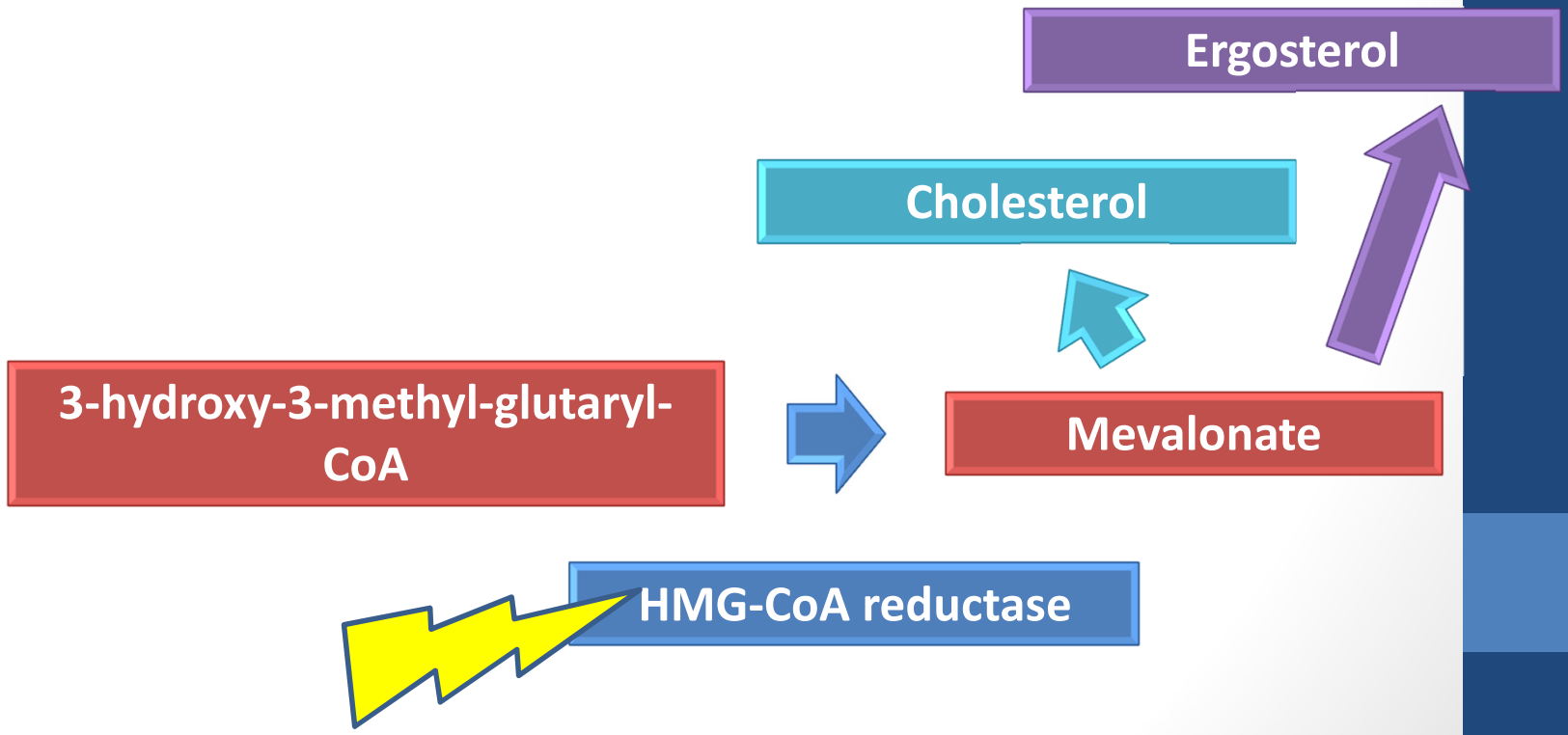
University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, Avda. Astrofísico Fco. Sánchez, S/N 38203 La Laguna, Tenerife, Canary Islands, Spain,[1] and Centre for Integrative Physiology, School of Biomedical Sciences, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, United Kingdom[2]

Received 9 March 2010/Returned for modification 13 May 2010/Accepted 10 September 2010

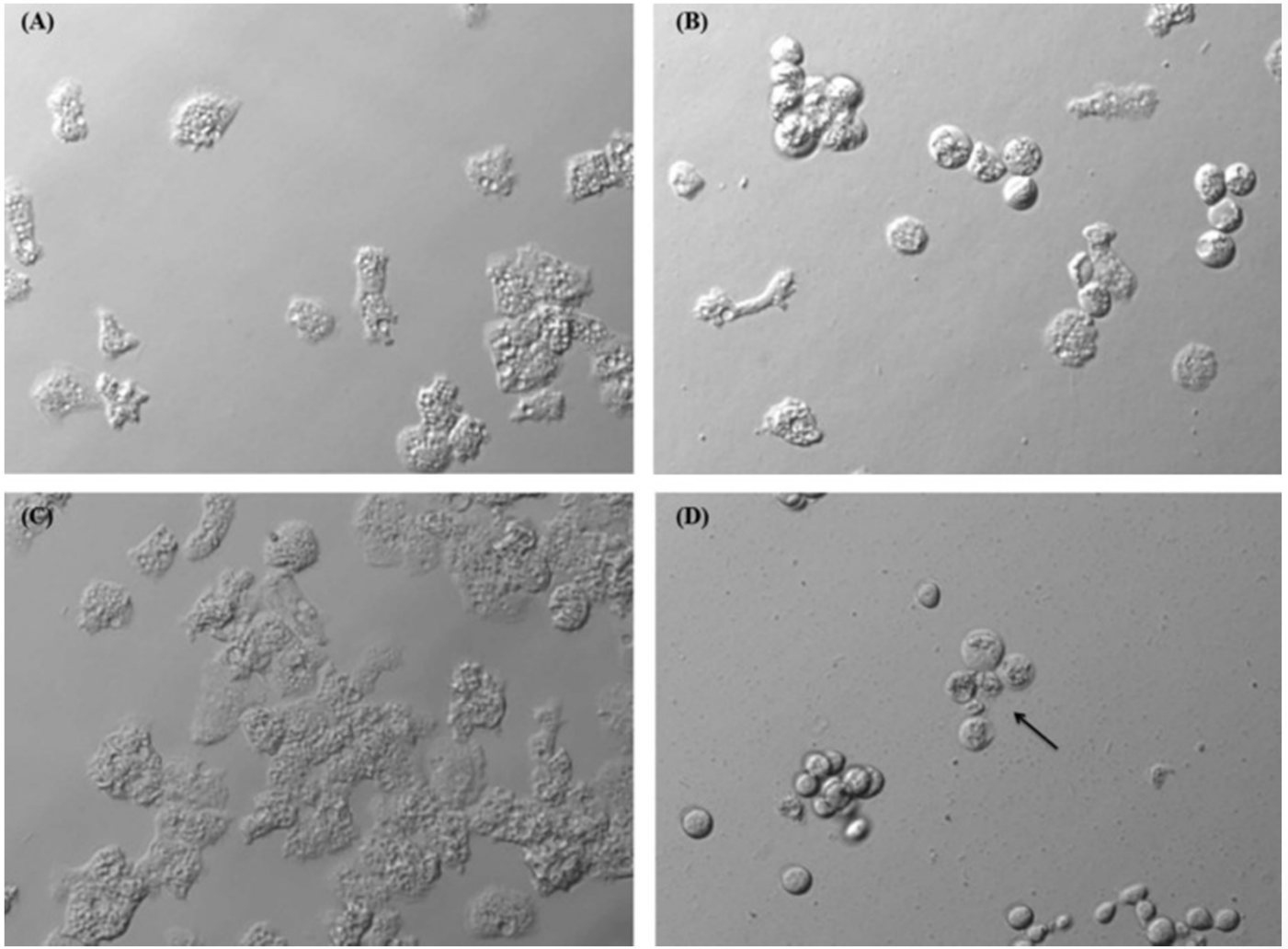
COMBINATION OF MOLECULAR THERAPY AND CHEMOTHERAPY

Sterol synthesis in amoeba

- Main sterol in the membranes→ ergosterol
- Same pathway as cholesterol synthesis in humans



siRNA HMCoA reductase



RNAi+→ non viable

Statins→ Inhibit HMCoA R

- Simvastatin
- Pravastatin
- Lovastatin
- Atorvastatin
- Fluvastatin

- Strains:
  - *Acanthamoeba castellanii* Neff (ATCC 30010)
  - Clinical strains:
    - CLC-16
    - CLC-41.r
    - CLC-51.l

Results

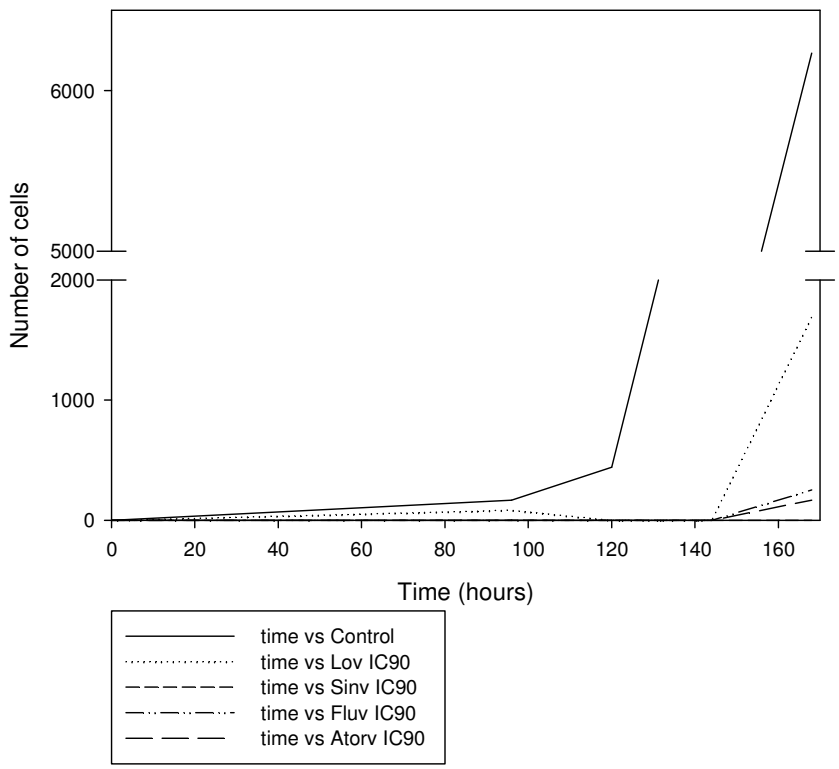
IC_{50} (mM) 96h	AcNeff	CLC-16	CLC-41.r	CLC-51.l
Lovastatin	17.14 ± 1.85	-	46.65 ± 4.50	-
Pravastatin	58.75 ± 11.01	-	-	-
Simvastatin	10.24 ± 1.09	31.44 ± 2.06	14.60 ± 0.59	39.73 ± 4.34
Fluvastatin	9.19 ± 0.98	54.64 ± 2.69	24.29 ± 0.97	16.50 ± 1.03
Atorvastatin	15.12 ± 2.19	33.34 ± 2.64	13.70 ± 0.81	26.63 ± 1.20

IC_{90} (mM) 96h	
Lovastatin	23.68 ± 0.32
Simvastatin	21.37 ± 1.51
Fluvastatin	20.70 ± 2.15
Atorvastatin	41.09 ± 0.00

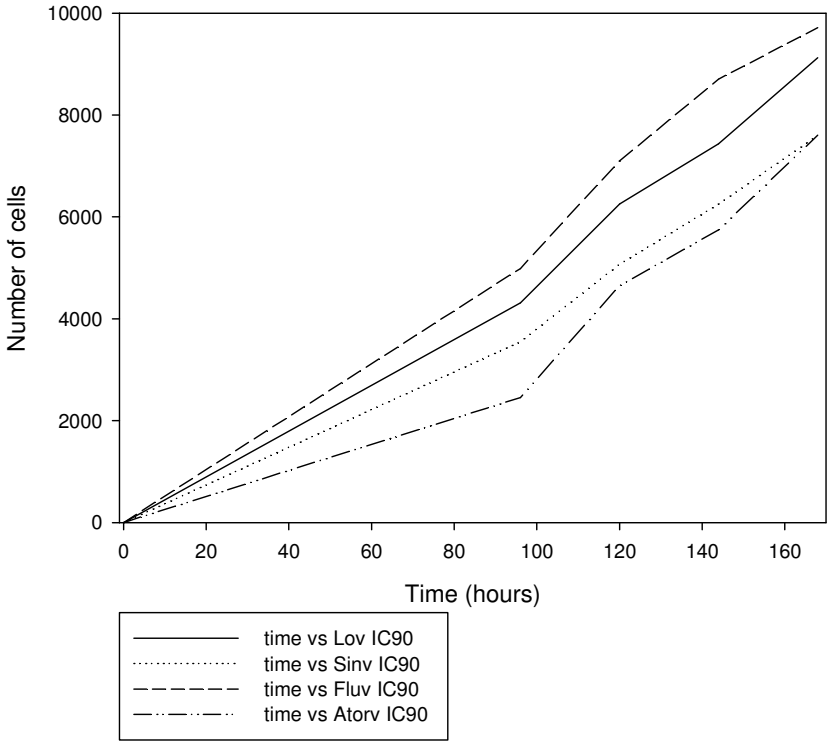
Results

→ Cysticidal activity /Toxicity

Trophozoites when IC_{90} is used



Unviable cyst when IC_{90} is used



Conclusions

- Statins are amoebicidal and cysticidal (10X lower humans)
- Future therapy?→ elucidated target using siRNA



Editor: Edlind | Section: Experimental Therapeutics | Designation: T

Inhibition of 3-Hydroxy-3-Methylglutaryl–Coenzyme A Reductase and Application of Statins as a Novel Effective Therapeutic Approach against *Acanthamoeba* Infections

Carmen María Martín-Navarro,[a] Jacob Lorenzo-Morales,[a] Rubén P. Machín,[b] Atteneri López-Arencibia,[a] José Manuel García-Castellano,[b] Isabel de Fuentes,[c] Brendan Loftus,[d] Sutherland K. Maciver,[e] Basilio Valladares,[a] José E. Piñero[a]

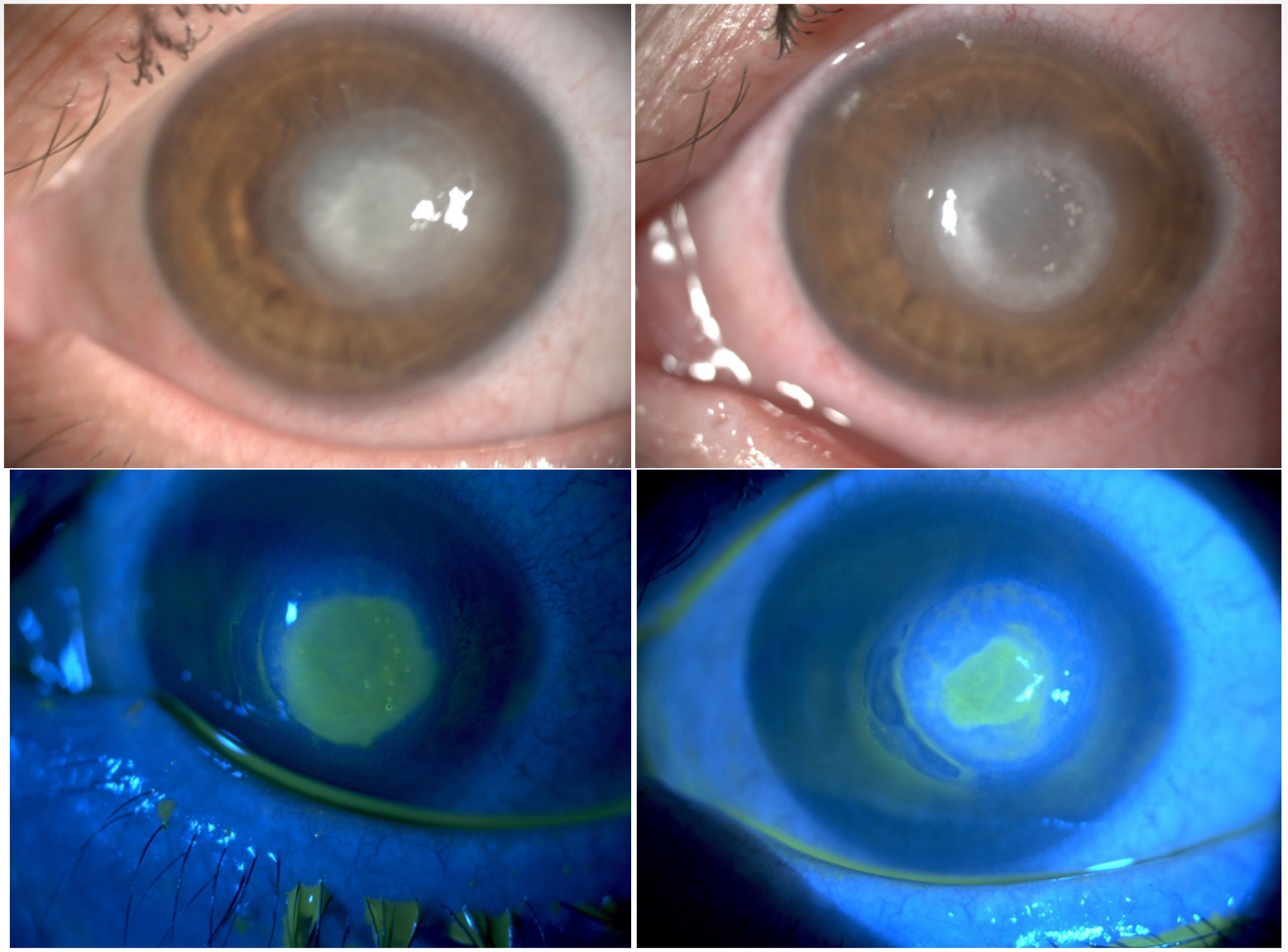
University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, La Laguna, Tenerife, Canary Islands, Spain[a]; Molecular Oncology Group (G-OncoMol), Research Unit, University Hospital of Gran Canaria Dr. Negrín, Barranco La Ballena, Las Palmas de Gran Canaria, Canary Islands, Spain[b]; Carlos III Health Institute, Microbiology National Center, Majadahonda, Madrid, Spain[c]; School of Medicine and Medical Science, Conway Institute, University College Dublin, Belfield, Dublin, Ireland[d]; Centre for Integrative Physiology, School of Biomedical Sciences, University of Edinburgh, Edinburgh, Scotland, United Kingdom[e]

Acanthamoeba **is an opportunistic pathogen in humans, whose infections most commonly manifest as *Acanthamoeba* keratitis or, more rarely, granulomatous amoebic encephalitis. Although there are many therapeutic options for the treatment of *Acanthamoeba*, they are generally lengthy and/or have limited efficacy. Therefore, there is a requirement for the identification, validation, and development of novel therapeutic targets against these pathogens. Recently, RNA interference (RNAi) has been widely used for these validation purposes and has proven to be a powerful tool for *Acanthamoeba* therapeutics. Ergosterol is one of the major sterols in the membrane of *Acanthamoeba*. 3-Hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase is an enzyme that catalyzes the conversion of HMG-CoA to mevalonate, one of the precursors for the production of cholesterol in humans and ergosterol in plants, fungi, and protozoa. Statins are compounds which inhibit this enzyme and so are promising as chemotherapeutics. In order to validate whether this enzyme could be an interesting therapeutic target in *Acanthamoeba*, small interfering RNAs (siRNAs) against HMG-CoA were developed and used to evaluate the effects induced by the inhibition of *Acanthamoeba* HMG-CoA. It was found that HMG-CoA is a potential drug target in these pathogenic free-living amoebae, and various statins were evaluated *in vitro* against three clinical strains of *Acanthamoeba* by using a colorimetric assay, showing important activities against the tested strains. We conclude that the targeting of HMG-CoA and *Acanthamoeba* treatment using statins**

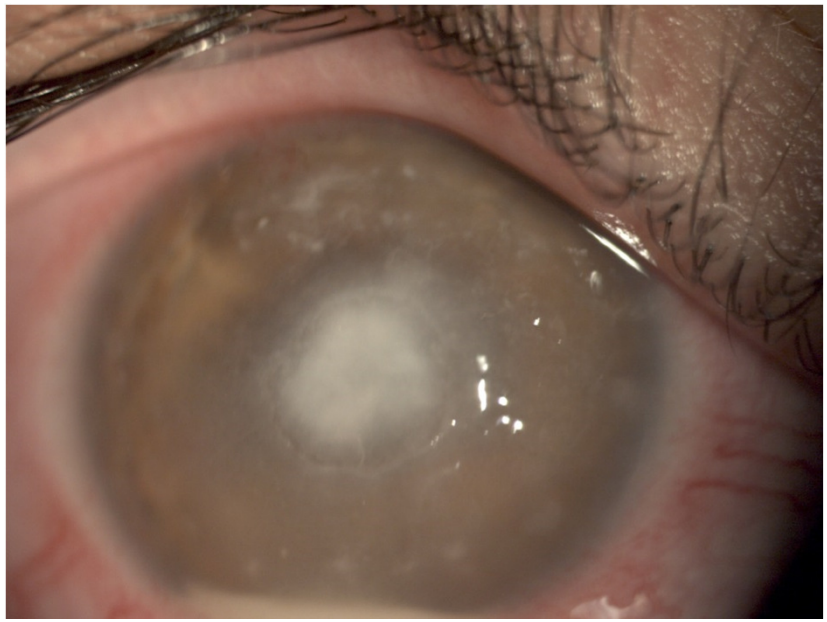
First trials in AK Patients

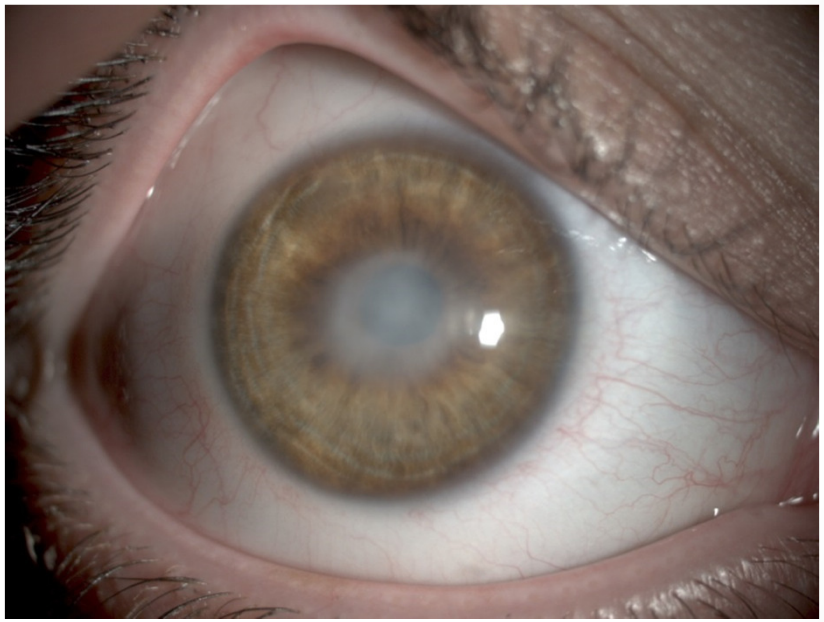


- March 2015-May 2015
- 4 patients not responding to AK therapy (chlorhexidine 0.02%)
- 2 Contact lens wearers and 2 non-contact lens wearers
- Once *Acanthamoeba* was diagnosed → atorvastatin 20mg



Patient 1





Patient 4

Acknowledgements

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ricet

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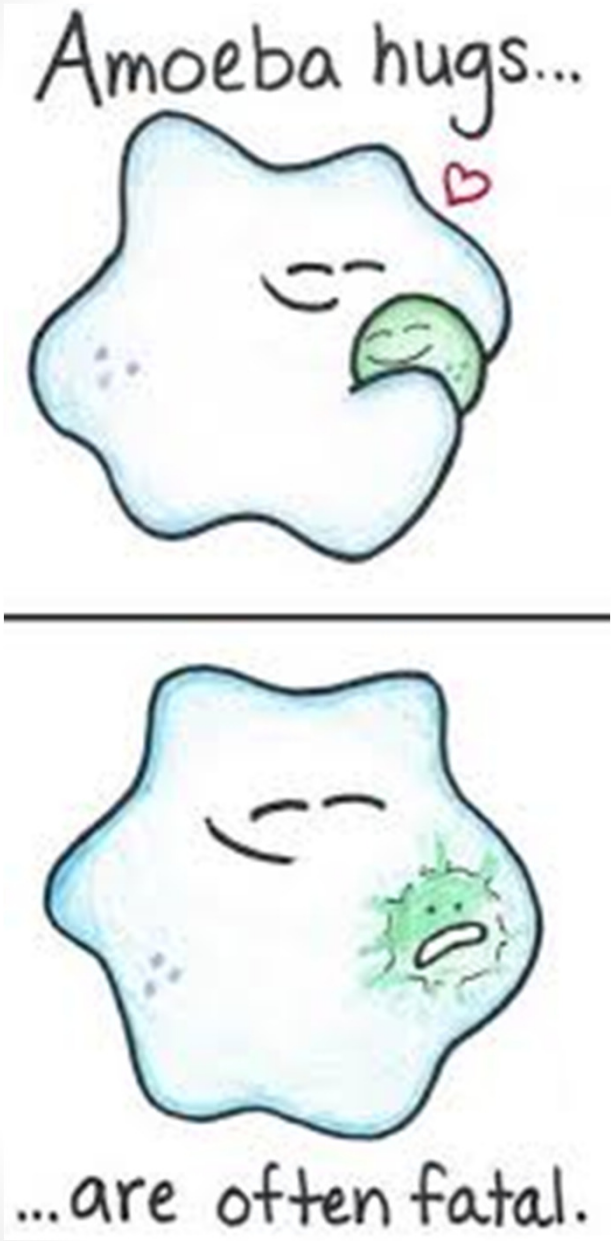
Free-Living Amoebae Team

We do also do some work!









THANK YOU!