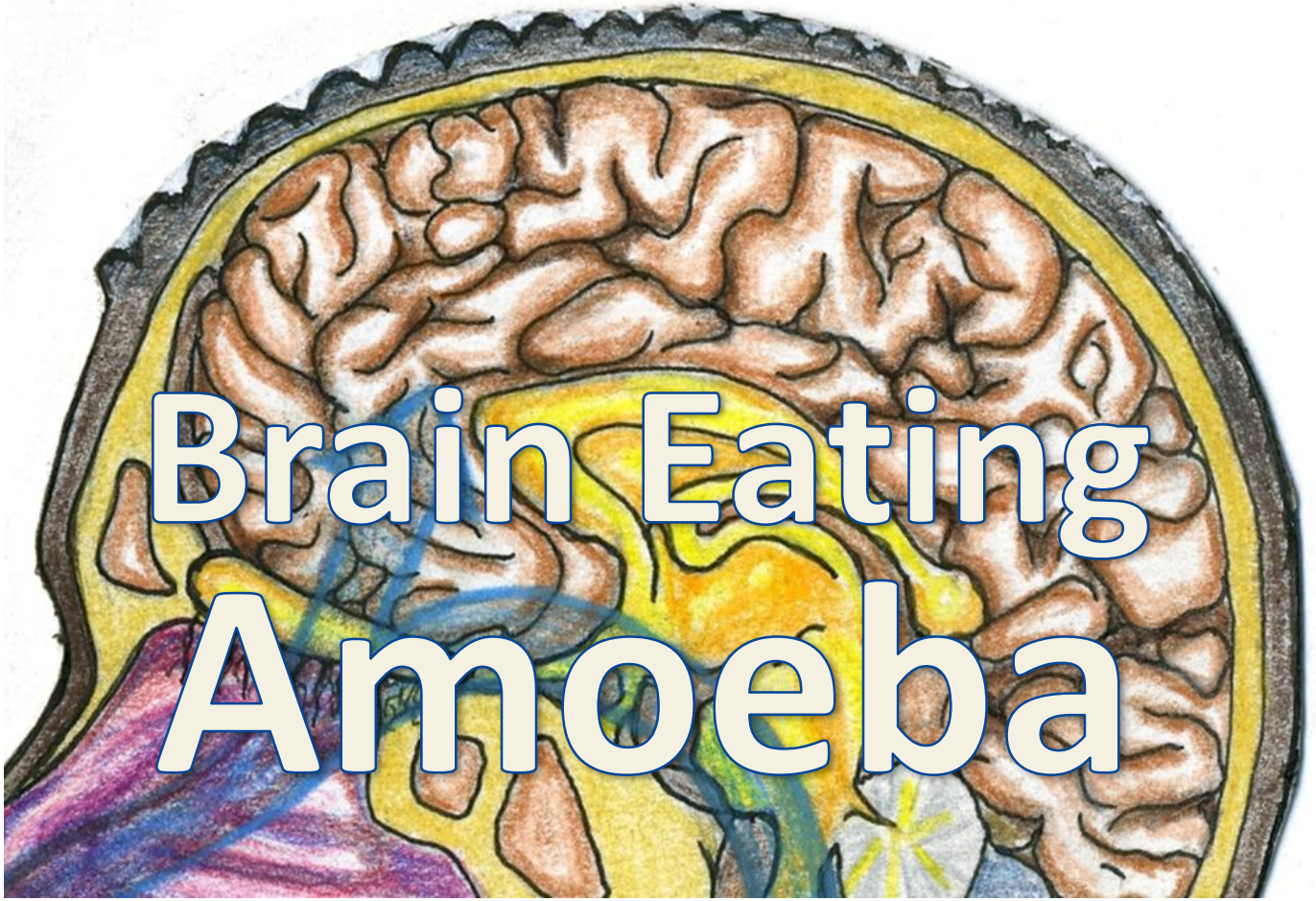
Isolation and Identification of the Brain Eating Amoeba *Naegleria fowleri*



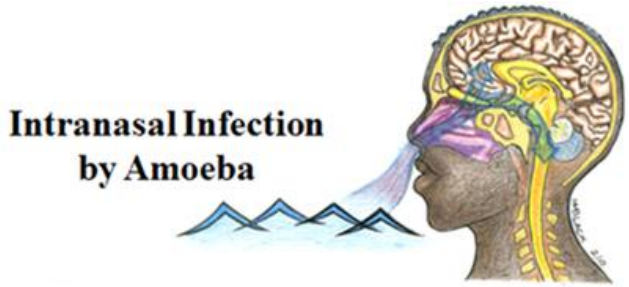
Research Simulation Case Study

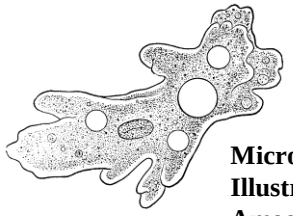
By Lycurgus L. Muldrow, Ph.D.

Introduction

Observation: The day after swimming, and having fun in a Florida river, a 16 year old girl, developed a severe headache and vomiting. The next morning, her fever rose to 104^0F, she became delusional and was then diagnosed with a *Naegleria fowleri* (*N. fowleri)* infection. Sadly, the girl died two days later from this ravaging infection that has no cure.

Introduction: N. fowleri is an amoeba, a single celled organism that can be found living free in very warm fresh water. Most species of amoebas are harmless to humans, but *N. fowleri* is a deadly pathogen (disease causing organism) that causes the disease called primary amebic meningoencephalitis (PAM). *N. fowleri* is acquired when the amoeba enters the nose, but not the mouth. In the United States, there have been 128 reported cases since 1962 and only one person has survived the infection.





Microscopic Illustration of Amoeba

Introduction

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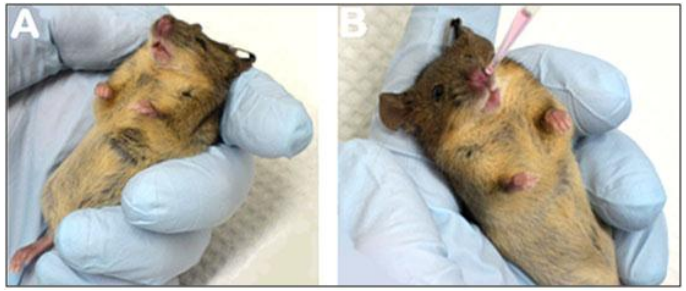
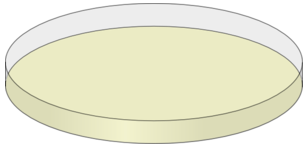
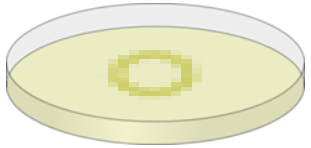
Background Facts:

- *N. fowleri* infections have been examined by studying infections in mice. This amoeba introduced into the nostril of a mouse migrates, by way of the olfactory nerve in the nose, to the brain. Within several days the brain tissue is destroyed and the mouse dies.
- In the environment *N. fowleri* survives by eating bacteria, but is present in very low numbers and is hard to find. In the laboratory N. *fowleri* grows well under controlled conditions in a petri dish when a bacteria food supply is added.
- *N. fowleri* in the environment grows in very warm waters, cool water kills it. In fact, it can grow in temperatures up to 46^0C (114.8^0F), which kills most other amoeba and protozoans.





Petri dish with bacteria.



The ring is millions of *N. fowleri* cells that have grown after one amoeba was placed in the center of this petri dish with bacteria.

Incubator with adjustable temperature settings. Petri dishes can be placed in it.

Introduction

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Research and Hypothesis:

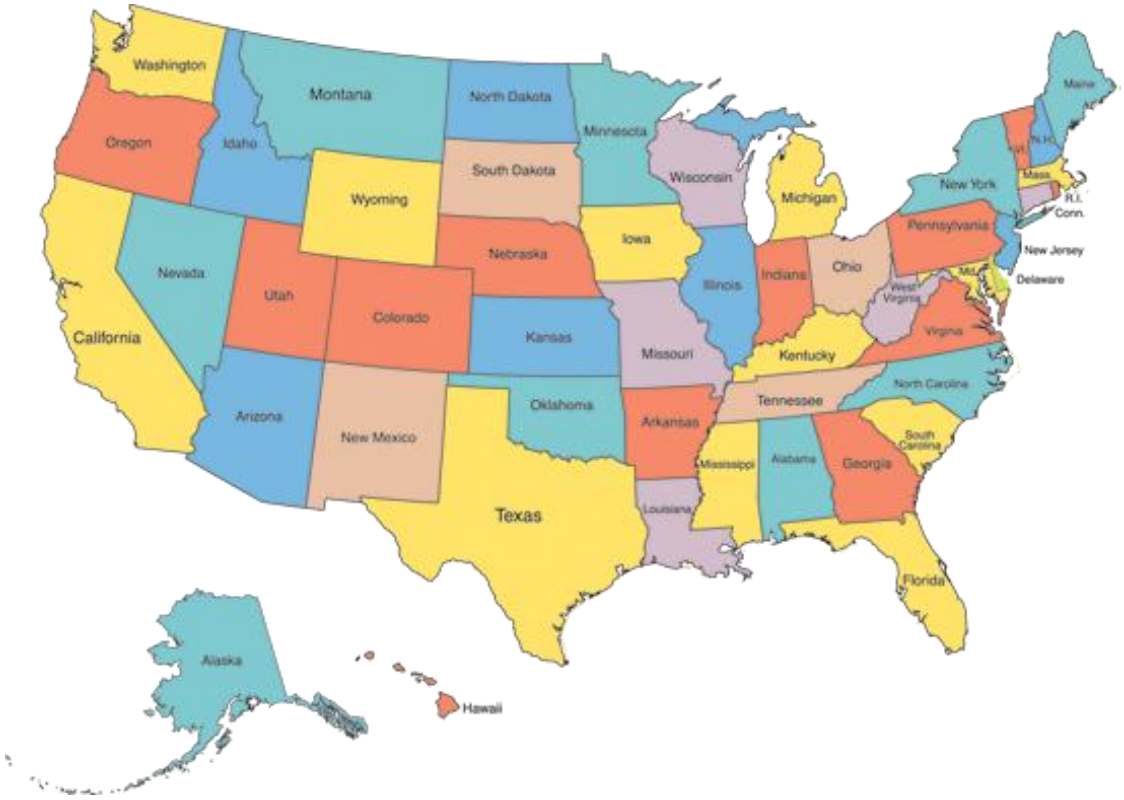
Suppose you are a professor and research scientist with a Ph.D., and you are employed at Washington University. In this position the Centers for Disease Control (CDC) awarded you a $2 million grant through your university to determine if *N. fowleri* is in lakes and rivers in 2 different states within the U.S. The goal of your research is to find which bodies of water are contaminated with *N. fowleri* so the population can be warned not to swim in that water. This four year grant pays part of your salary, the salary of two technicians with masters degrees, travel and supplies for the research.

Your working hypothesis is: if *N. fowleri* present in a body of water, then by developing the right experimental protocol (i.e. method) *N. fowleri* can be isolated from water samples, grown in the laboratory and tested for pathogenicity.

Materials and Methods

Experimental Protocol Development:

1. Which two states within the U.S. would you conduct your research and look for *N. fowleri*, and why?



Materials and Methods

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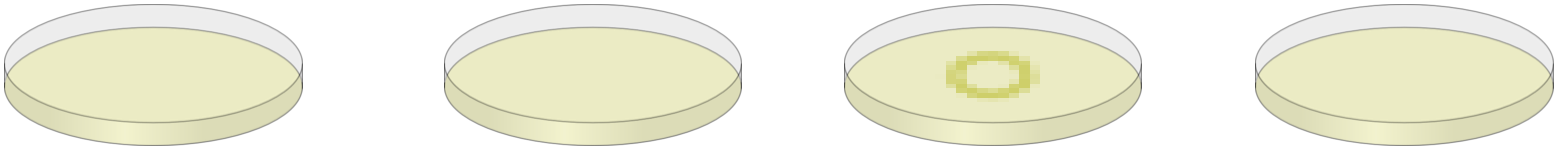
1. In the development of your experimental protocol, you have broken it down into 3 different stages: first, isolate *N. fowleri*; second, grow it in the laboratory; and third, conduct further identification experiments to determine if the isolated amoeba may be *N. fowleri*.
    1. *Isolate*: Your strategy for finding *N. fowleri* is to take samples of water from lakes and rivers, and then eventually test these samples to see if *N. fowleri* is present. How many water samples would you take from each lake or river and why?
    2. Grow *N. fowleri*: Write an experimental protocol you would use to try to selectively grow *N. fowleri* from the waters samples you have collected.
    3. *Further Identification*: If an amoeba grew in the laboratory from step 2 above, write an experimental protocol you could use to further determine if these cells might be *N. fowleri*, or just some other amoeba.

Results

1. Suppose you are in your third year of conducting your research project and you have so far taken water samples from 543 different lakes and rivers. Below are the results from just four samples taken from site 543 after you have completed stage 2 (Grow *N. fowleri*) of your protocol. What would your conclusion be for each of the four samples below?



Sample A Sample B Sample C Sample D

2. Would you conduct stage 3 of your protocol (Further Identification) on any or all of the above samples, and if so why?

Discussion

You are now in your fourth and final year of your funded research project. You have tested 673 lakes and rivers in two different states. With your experimental protocol that selectively grows *N. fowleri*, you identified three samples that produced a ring of cells that resembled what *N. fowleri* cells look like when grown in control conditions in a petri dish with bacteria (see slide 3 for control). In addition, you did further studies to identify if these three samples may be composed of *N. fowleri*. Upon conducting further identification experiments, two of these three samples gave positive results.

Write a brief discussion about these two samples that gave positive results in your further identification experiments. In your discussion indicate if you identified the *N. fowleri* species with a 100% certainty, or do you simply have strong evidence that *N. fowleri* may be present in these samples. Would you recommend that CDC warn people not to swim in these two bodies of water that gave positive results?

Finally what recommendation would you give CDC concerning the one sample that produced a ring of cells in the petri dish, but gave negative results in the further identification experiment?