NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

A Bioinformatic Investigation of a Mysterious Meningoencephalitis

by
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Part I – Investigating Emmanuelle's Meningoencephalitis with BLAST (Generating a Hypothesis)

The following story is adapted from a real medical case from Chomba et al. (2017). Some patient information has been altered in the presentation below.

Emmanuelle was a 14-year-old boy who lived in the Kafue district of the southern part of Lusaka province, Zambia. He was admitted to the University Teaching Hospital in Lusaka, presenting with sudden onset of seizures and fever on admission. On physical examination, he was febrile, comatose, and had a stiff neck. Emmanuelle's parents told the doctors that he had not traveled anywhere or done anything special, except for a swim with his classmates over the weekend in the Kafue River, the longest river lying wholly within Zambia. A working diagnosis of severe septicemia with acute meningoencephalitis was made by the doctors, and Emmanuelle was started on IV ceftriaxone twice daily. To discover the causative agent of the infection, doctors collected Emmanuelle's cerebral spinal fluid (CSF). Initial analysis unfortunately did not reveal any organism from microscopy or culture, but did show elevated white blood cell count. Because of the elevated white blood cell count, doctors were confident that Emmanuelle was suffering from an infection. To ascertain what microorganism was rampaging through his body, the doctors performed a second lumbar puncture on Day 3 after admission. From the obtained spinal fluid, clinical scientists were able to purify a fragment of foreign DNA as shown below:

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                                  atgtcctc ccaacacatc
aatatctgcg gcaagttcgg tccagagatg gacaagactg ttcaagccat gattgctaga
ggcaagggtc ttttggctgc cgatgaatcg acatcaacca ttggaaagcg ttttgaaaag
atctctttgg agaacaatga aaccaatcgt caagcctatc gtgaattgct cttcactgct
ccaaaggaat acactcaata catcagtggt gtgattttgt atgaagagac tttgttccaa
tcgactctca gtggaaagcc attcgctgaa ctcttaaccg aggctggagt tgttccaggt
attaagcttg atttgggtgt aaagaatttg ccaggtactg atggtgagca agccactcaa
ggtttggatg atttggagaa gagaattaag aagtactatg aaagaggtgc tcgtttcgcc
aagtggagag ctgtctacaa gatcaacgac agaggattgc catctcaact tgcagtcgat
caaaatgctg aaactttggc cagatacgca gccatttgtc aagagaatgg tctcgtgcca
attgttgaac ctgaaatttt ggtcatggaa ggatctcaca atattgaagt ctctttgcaa
gtcaccaaga gagttttggc tgccgtcttc caacgattga ttcaacacaa tgtcgatttg
aatcgaatca tcttgaagcc aaacatggtc ttgccaggta acgattgtcc aaccaagact
gaagatgata agaagattgc tcaatacact gttgatgctt tgactagcac tgtcccacct
gcagtgagag gaattatgtt cttgtctggt ggtcaatcgg agaagcaagc tgttgagact
ttgaatgaaa tcaacaaggt caatgctcca aagccatggg ctctctccta ctcttatggt
agagctttgc aagattccgc aatcaagact tggcaaggaa agaaggaaaa tgttgaagca
gctcaaaaag cttacattga acaagccaag aagtgctcat tggcttccaa gggcgagctc
                                  taa
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Questions

1. Perform a standard nucleotide BLAST (https://blast.ncbi.nlm.nih.gov) using the isolated DNA fragment shown on the previous page of this handout (be sure to copy the entire sequence, starting with **`atgtcctc`** and ending with **`taa`**). Based on your results, what do you think could be the cause of Emmanuelle's meningoencephalitis? Explain your answer with evidence by showing a screenshot of your BLAST result and citing BLAST statistics such as E-Value, Max Score, etc.

2. Speculate on how Emmanuelle might have acquired the infection. How common is the infection? Is there any effective treatment? What is the prognosis of this infection? (*Suggested resource:* Centers for Disease Control and Prevention website.)

3. Is this microorganism a eukaryote or a prokaryote? Explain.

4. Use NCBI ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/) to identify all possible open reading frames from the above DNA sequence by pasting it into the query box. How many different open reading frames do you see using the default setting? Why so many? Show a screenshot of the ORFfinder result as evidence.

5. Which open reading frame is likely to encode a protein? Explain your answer.

6. Verify your answers to Question 5 by using PROTEIN BLAST (https://blast.ncbi.nlm.nih.gov) to identify the protein encoded by the isolated DNA fragment. Explain how you achieved this. Show a screenshot of the BLAST result as evidence.

7. Briefly describe how you may use polymerase chain reaction (PCR) to verify the identity of the pathogen infecting Emmanuelle. Focus on the flow or logistics of the experiment rather than on specific details, such as the amount and the concentration of the reagents.

Part II – Molecular Investigation of Emmanuelle's Meningoencephalitis (Experimental Validation)

Despite receiving antibiotic treatment, Emmanuelle's condition deteriorated. He developed high fevers with generalized seizures and depression of consciousness, and required ventilatory support. A contrast-enhanced computed tomography scan showed brain swelling and hemorrhage, which indicated that his meningoencephalitis had worsened. A direct wet mount microscopic examination of a third CSF sample collected on Day 8 after admission was finally productive and showed several highly motile amoebic trophozoites and cysts. The most common cases of amoebic encephalitis in the Kafue district were due to *Naegleria fowleri* or *Acanthamoeba* spp. To confirm the identity of the "brain eating" amoeba, clinical scientists performed a polymerase chain reaction (PCR) by amplifying the ribosomal small subunit (SSU) 18S rRNA gene as seen in Figure 1; the final PCR results are shown further below in Figure 2.

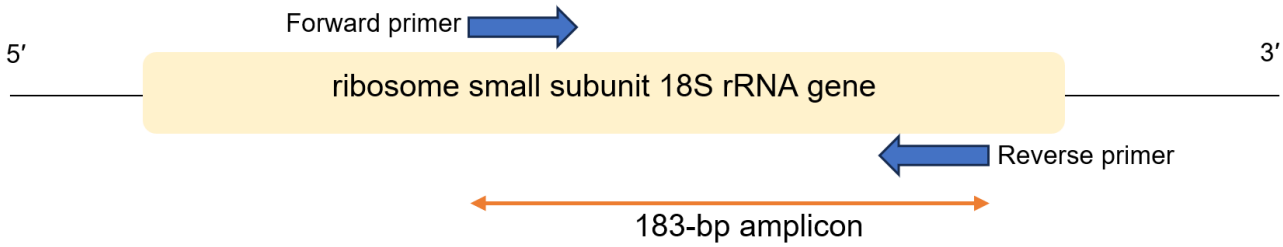

Figure 1. Simplified diagram of primer binding sites and expected product size for PCR.

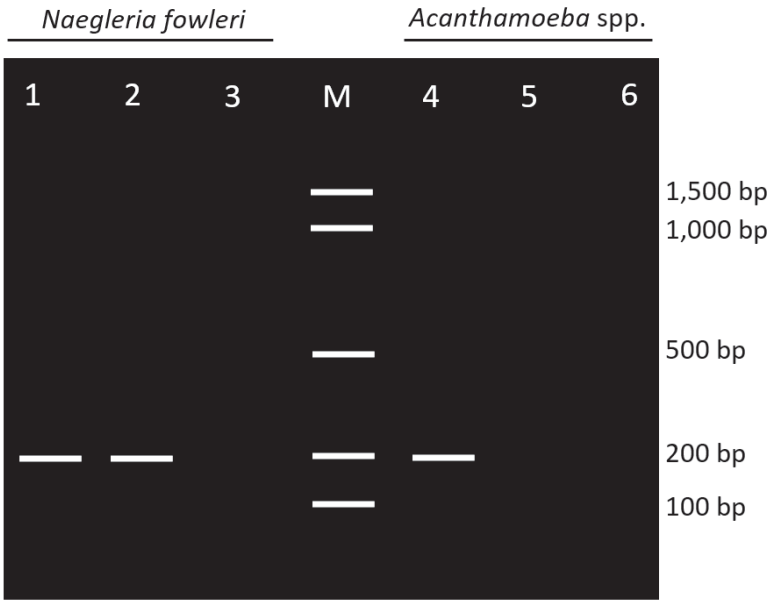

Figure 2. Final PCR results (based on Su et al., 2013):

- Lane 1: *N. fowleri* Carter strain
- Lane 2: Emmanuelle's CSF sample
- Lane 3: Non-pathogenic *Naegleria* spp.
- Lane M: DNA ladder
- Lane 4: *A. castellanii* Neff strain
- Lane 5: Emmanuelle's CSF sample
- Lane 6: Non-pathogenic *Acanthamoeba* spp.

Questions

8. List and explain the necessary reagents needed to perform a PCR reaction.

9. List and explain the necessary steps involved in a PCR reaction.

10. Based on the above data, explain how the PCR test was visualized in Emmanuelle's case. Instead of the method used in this case, can you recall any other ways one can "visualize" a PCR test result? (*Hint:* think about the COVID test.)

11. In the PCR test, identify the negative and positive controls.

12. Based on the result, which organism was responsible for Emmanuelle's encephalitis? Explain your decision.

13. Explain how missing Lane 1 would affect the test result interpretation. Assume all other lanes were available and unchanged.

14. Explain how missing Lane 2 would affect the test result interpretation. Assume all other lanes were available and unchanged.

15. Explain how missing Lane 3 would affect the test result interpretation. Assume all other lanes were available and unchanged.

16. Explain how missing Lane M would affect test result interpretation. Assume all other lanes were available and unchanged.

17. Explain how missing Lanes 4–6 would affect test result interpretation. Assume all other lanes were available and unchanged.

18. If you want to further confirm the cause of Emmanuelle's infection, what other experiments (both dry and wet laboratories) can you do with the PCR amplified DNA?

Part III – Prognosis and Prevalence of *N. fowleri* Meningoencephalitis (Epidemiological Survey)

With the confirmation of *N. fowleri* in Emmanuelle's CSF, amphotericin B was administered, but although this treatment was started immediately, Emmanuelle remained comatose. After 21 days of hospitalization, his health condition drastically deteriorated despite the best efforts of his medical team. On Day 25, Emmanuelle sadly passed away from severe shock, followed by primary amoebic meningoencephalitis.

Questions

19. Why was amphotericin B used to replace ceftriaxone treatment?

20. Even with the right treatment, the patient passed away. Suggest three reasons to explain why amphotericin B would be ineffective in Emmanuelle's case.

21. The Centers for Disease Control and Prevention (CDC) reported the number of cases of primary amoebic meningoencephalitis by age group and gender from 1962 to 2022, as shown below (Figure 3). What trends do you observe? Hypothesize the reasons for the observed trends. (You should name and explain two trends.)

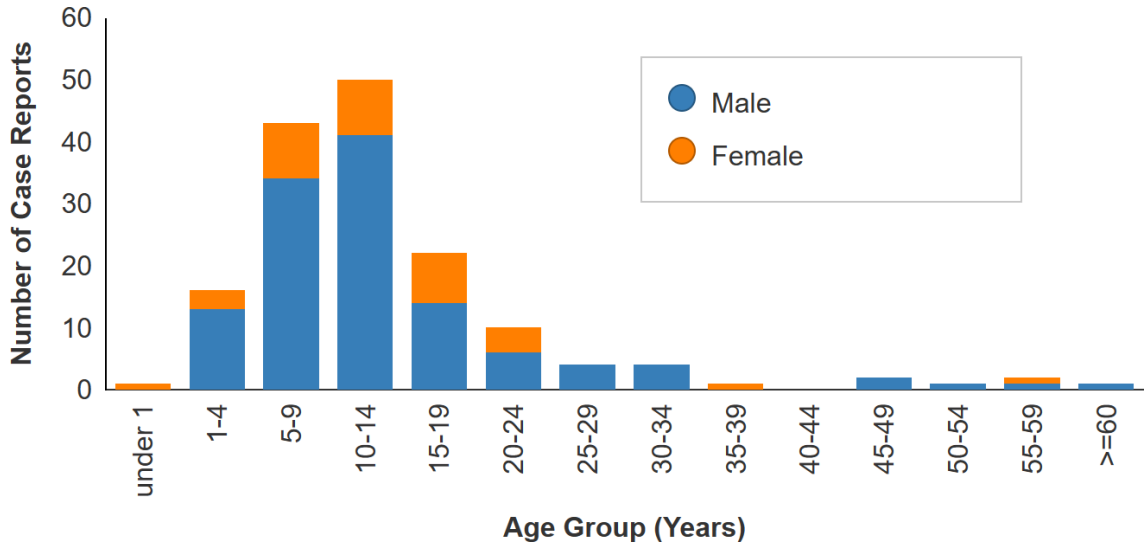

Figure 3. Number of case reports of primary amebic meningoencephalitis by age group and gender: United States, 1962–2022 (N = 157; median age = 11; 77.1% male). *Credit:* CDC, https://www.cdc.gov/parasites/naegleria/graphs.html.

22. The CDC reported the number of cases of primary amoebic meningoencephalitis by month of illness onset and probable water exposure from 1962 to 2022 (Figure 4). What trends do you observe? Hypothesize the reasons for the observed trend. (You should name and explain two trends.)

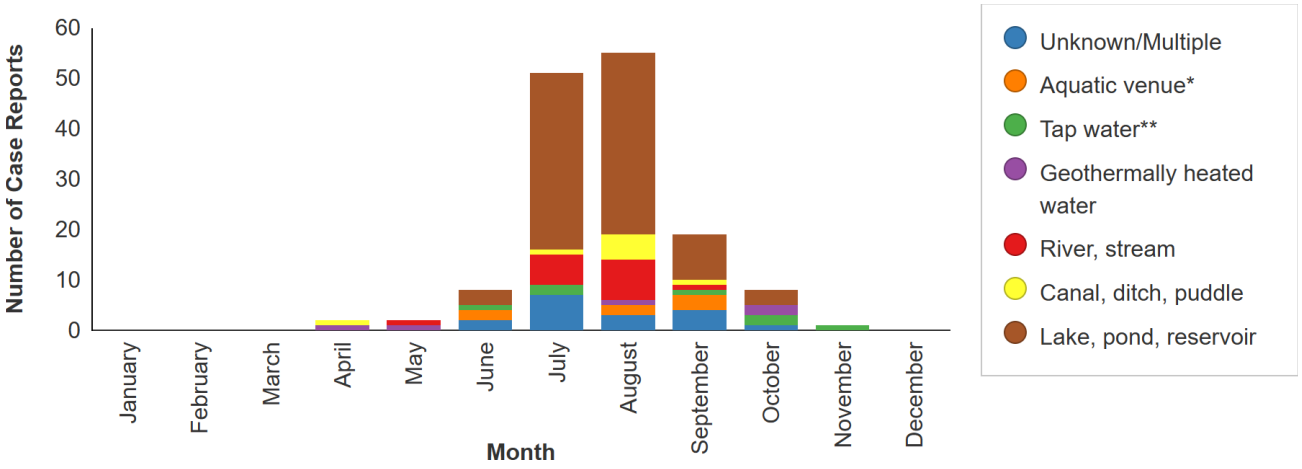

Figure 4. Number of case reports of primary amebic meningoencephalitis, by month of illness onset and probable water exposure: United States, 1962–2022 (N = 146). Month of illness onset unknown for 11 cases. Of those case reports missing the month of exposure, probable water exposures included lake, pond, reservoir (N =5), unknown/multiple (N=5), and geothermal water (N=1) *Aquatic venues are artificially constructed structures or modified natural structures where the general public is exposed to water intended for recreational or therapeutic purpose (e.g., swimming pools, splash pads, hot tubs, etc.). **Water was forced up the nose during use. *Credit:* CDC, https://www.cdc.gov/parasites/naegleria/graphs.html.

23. The CDC reported the number of cases of primary amoebic meningoencephalitis by state of exposure from 1962 to 2022 (Figure 5 below). What trends do you observe? Hypothesize the reason(s) for the observed trend.

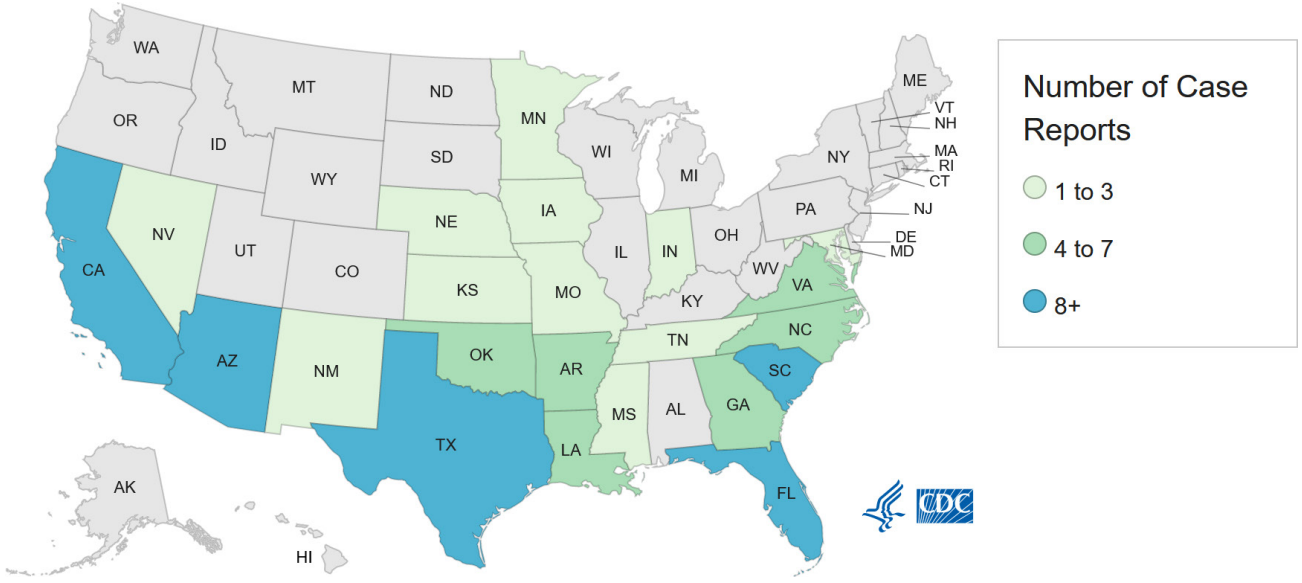

Figure 5. Number of case reports of primary amebic meningoencephalitis by state of exosure: United States, 1962–2022 (N = 157). *Credit:* CDC, https://www.cdc.gov/parasites/naegleria/state-map.html.

Part IV – Protein Modeling and Drug Design (New Treatments)

Infectious diseases caused or transmitted by parasites are worldwide public health issues, particularly in underdeveloped countries. Primary amoebic meningoencephalitis is a fast onset and often overlooked parasitic disease, resulting in late diagnosis and high mortality. Amphotericin B is a generic antifungal medication that is not specific in treating *N. fowleri* infection and is only effective when administered early in the course of infection. As a result, a more targeted approach that is specific to *N. fowleri* is needed. Rational drug design makes use of the bioinformatical power currently available. For diseases like *N. fowleri* infection, this approach attempts to identify a biomolecular target (often a protein) that is essential and specific for the infectious agent while having little or no effect on the host. This target is then used in the search for compounds that impair its function. Once a lead compound is identified, it can then be used as a starting point in the drug optimization process (Méndez, 2019).

Rational drug design is much easier when three-dimensional information about the structure of a target protein is available. High-resolution imaging of biomolecules can often be obtained from techniques such as X-ray crystallography and NMR spectroscopy. This particular approach to drug discovery is sometimes referred to as structure-based drug design. A good example of an approved drug developed through structure-based drug design is dorzolamide, a carbonic anhydrase inhibitor used to treat glaucoma. In Part I of this case study, you identified the protein encoded by the DNA fragment isolated from Emmanuelle's spinal fluid. In this section of the case, you will explore whether the protein from *N. fowleri* could serve as a target for rational drug design to treat amoebic meningoencephalitis.

Questions

24. Based on your answer to Question 6, do humans possess a homolog of this protein? Do you expect the *N. fowleri* protein would function in the same way as the human protein? Why?

25. How would the similarity/difference in the protein structure/function (from Questions 6 and 24) between *N. fowleri* and human aldolase affect the rational drug design process?

26. Use Protein Data Bank (PDB, https://www.rcsb.org/) to search for the existing structure of this *N. fowleri* protein. If you do not find it in PDB, then build a structural model of this protein by using SWISS-MODEL9 (https://swissmodel.expasy.org/interactive) using the following steps:
    a. Copy and paste the amino acid sequence into the query box and click "Build Model."
    b. Wait 5–10 minutes for the program to build the model. *After the model is built, take a note of the template protein and organism the program used to build the model along with the sequence identity* (located in the left panel labeled *"Template"*). As part of the SWISS-MODEL output, you may see a model built based on Google's alpha-fold. For our purposes, please disregard the alpha-fold model. Take a screenshot of the result to be used later (Question 27 below).
    c. Download the model by clicking on "Model 01" and then "PDB format." Open the downloaded model structure with PyMOL.
    d. In the same PyMOL session, fetch human counterpart protein and superimpose the model and human protein.
    e. Based on the structural comparison, discuss the similarities and differences between the two proteins at secondary, tertiary, and quaternary levels. Show screenshots of superimposed structures.

27. Based on your observation from Question 26 (b), hypothesize how SWISS-MODEL predicts unknown protein structure. Show a screenshot of the result.

28. To assist your further analysis, you should also perform a protein sequence alignment (https://www.ebi.ac.uk/Tools/msa/clustalo/) of the *N. fowleri* protein and human counterpart protein. What are some similarities and differences? Pay specific attention to the enzyme active site. Show the sequence alignment picture. (Note that protein sequence alignment requires the amino acid sequence to be in the FASTA format, which means the sequence input should begin with ">Name of your sequence", and your actual sequence comes in on the second line. See this short video tutorial for how to use: *Aligning Sequences Using Clustal Omega* produced by Dr. K' teaches, 2015. Running time: 6:13 min. <http://youtu.be/zlnDEzqjCPA>.

29. With the sequence alignment and superimposed structures ready, revisit your answers to Questions 24 and 25, and discuss whether the *N. fowleri* protein isolated from Emmanuelle's spinal fluid would be a good target for rational drug design. If yes, explain why and how; if no, briefly propose another approach for rational drug design that targets *N. fowleri*.

References

Centers for Disease Control and Prevention (CDC). (2023). *Naegleria fowleri* | Primary amebic meningoencephalitis (PAM) — Amebic encephalitis [webpage]. <https://www.cdc.gov/parasites/naegleria/>

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