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ABSTRACT

MELANIE A. MOSER
A Descriptive Review of *Balamuthia* and Non-Keratitis *Acanthamoeba* Cases in the United States, 1955-2009
(Under the direction of Richard Rothenberg, Professor)

Free-living amebae are ubiquitous in the environment and occasionally invade and parasitize host tissues causing illness in humans. Despite possibly frequent exposure to these organisms, infection is rare and why some people, healthy or not, end up with illness and others do not is still unclear. Human infections are rare; when illness does occur, it is often fatal. Only two papers have examined data from the literature and cases reported to the Centers for Disease Control and Prevention, and both were published over twenty years ago. The purpose of this study is to better document the epidemiology of *Balamuthia* and non-keratitis *Acanthamoeba,* give insight into trends of these infections over time, and contribute to the scientific and medical community by producing the only comprehensive review of all *Balamuthia* and non-keratitis *Acanthamoeba* cases in the United States from 1955 through 2009. This study also examines cases that have survived in an attempt to determine if there is evidence for the effectiveness of a particular treatment regimen. Only a small number of patients have survived these infections, so any evidence for a successful course of treatment could be crucial for future cases.

INDEX WORDS: free-living amebae, *Balamuthia mandrillaris, Acanthamoeba* spp., non-keratitis *Acanthamoeba*

A DESCRIPTIVE REVIEW OF *BALAMUTHIA* AND NON-KERATITIS *ACANTHAMOEBA* CASES IN THE UNITED STATES, 1955-2009

by

MELANIE A. MOSER
B.S. FROSTBURG STATE UNIVERSITY

A Thesis Submitted to the Graduate Faculty
of Georgia State University in Partial Fulfillment
of the
Requirements for the Degree

MASTER OF PUBLIC HEALTH

ATLANTA, GEORGIA
2011

APPROVAL PAGE

A DESCRIPTIVE REVIEW OF *BALAMUTHIA* AND NON-KERATITIS *ACANTHAMOEBA* CASES IN THE UNITED STATES, 1955-2009

by

MELANIE A. MOSER

Approved:

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April 13, 2011
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Date

DEDICATION PAGE

This thesis is dedicated to all those who have suffered from and succumb to free-living ameba infections. May this work, in some small way, help to illuminate what is known and what further work needs to be done to ensure others have improved outcomes.

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I would like to thank Dr. Govinda Visvesvara, head of the Free-Living Ameba Laboratory at the Centers for Disease Control and Prevention, and his lab assistant, Mrs. Rama Sriram. Without the diagnostic work performed by the FLA Lab, efforts to investigate cases sent to the lab, maintenance of historical files, and Dr. Visvesvara's long and precise memory, this thesis could not have been undertaken. Dr. Visvesvara has worked tirelessly for over 30 years to improve diagnosis of free-living ameba infections and contribute to a greater understanding of the organisms; it was a sincere pleasure and honor to have his assistance on this project. I would also like to thank the Waterborne Disease Prevention Branch's (WDPB) Dr. Sharon Roy and Mr. Jonathan Yoder for their encouragement of this project and their willingness to serve as thesis committee member and practicum preceptor respectively. Dr. Roy was exceedingly patient and helpful in reviewing this document multiple times. Her suggestions on how to examine the data were crucial to this work. The practicum experience was foundational to the consolidation of some of the data explored in this thesis and proved to be an invaluable learning experience in how informal surveillance systems are developed and implemented.

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convinced a descriptive study was important enough to undertake, but our after-class conversations made me a believer. Although they were not involved in the thesis process, both Professor Russell Toal and Dr. Frances McCarty are exceptional faculty. Professor Toal was an inspiration in my first semester of graduate studies, showing real commitment to public health and social justice. Dr. McCarty was a wealth of patience and practicality in the biostatistics courses I had with her. Although I'll never be a statistician, she is the proof of why they are so needed in public health.

Lastly, I would like to thank my husband, Greg McNamara, for his encouragement and support in my decision to return to school to pursue a graduate degree. Public health is my passion and I thank him for his patience and willingness to listen as I went through this course of study and for his keen eyes in reviewing this document for errors.

AUTHOR'S STATEMENT

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CHAPTER I: INTRODUCTION

Background

Free-living amebae are ubiquitous in the environment and occasionally invade and parasitize host tissues causing illness in humans.[1,2] Although human infections are rare, when illness does occur, it is often fatal. There are four genera of free-living amebae known to infect humans.[2] *Naegleria fowleri*, which can cause primary amebic meningoencephalitis (PAM), is described in the media as the "brain-eating ameba"[3,4] and news reports of infections with this organism have likely raised awareness of free-living amebae. Other free-living amebae, such as *Balamuthia mandrillaris* and *Acanthamoeba* spp. can cause granulomatous amebic encephalitis (GAE), an infection of the central nervous system. Although there is only one species of *Balamuthia* known to infect humans, *B. mandrillaris*, there are multiple species of *Acanthamoeba,* at least eight, that can cause human infection. Acanthamoebiasis and balamuthiasis can manifest as disseminated infections throughout the body or localized in an area of the body, often affecting the brain, skin, and/or sinuses, as well as other organs such as the lungs, liver, kidneys or lymph nodes, either independently or in combination. *Acanthamoeba* spp. can also cause keratitis, which may threaten an infected person's vision by causing ulceration of the cornea, diminished sight, or even blindness. *Sappinia diploidea* also has been identified as the cause of one reported case of encephalitis.[1]

Naegleria fowleri has been isolated from warm freshwater, geothermal waters, soil, sewage, and unchlorinated or minimally chlorinated swimming pools.[5,6] Likewise *Acanthamoeba* spp. have been found in soil, sewage, swimming pools, and freshwater but

have also been identified in hot tubs, sea or brackish water, heating/ventilating/air conditioning (HVAC) units, dialysis machines, contact lens solutions, and intra-uterine devices (IUDs).[6] *Balamuthia mandrillaris* is found in soil.[7,8] *Balamuthia* might also be present in freshwater; there have been reports of *Balamuthia* GAE in dogs that swam in ponds but there have been no reported human cases where the only potential exposure was swimming.[9,10] Because these amebae naturally inhabit so many locations, it is likely that humans are frequently exposed to them. Immunologic studies of *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris* have shown that some humans have antibodies to them.[1] Two serologic studies carried out in the United States showed that persons who had extensive contact with freshwater lakes had antibody response to *N .fowleri*.[11] Antibodies to *B. mandrillaris* and *Acanthamoeba* spp. have been demonstrated in healthy as well as hospitalized persons.[1] Despite possibly frequent exposure, infection is rare and why some people, healthy or not, end up with illness and others do not is still unclear.

Culbertson was the first to suggest that free-living amebae could possibly cause infection in humans and showed pathogenicity using animal models.[2,12] Butt described the first U.S. cases of free-living ameba infection, PAM caused by *N. fowleri*, in 1966.[13] Less than ten years later in 1972, Jager and Stamm reported the first human case of *Acanthamoeba* disease, which occurred in the U.S, although it was another eight years before it was definitively recognized that *Acanthamoeba* was the causal agent not *Hartmannella* as was supposed in the original case report.[14,15] Eventually several reports preceding the 1966 first report of free living ameba infection were retrospectively identified as cases of *Acanthamoeba* spp. as understanding of the organisms increased.[2] Recognition that *Balamuthia* (referred to as leptomyxid ameba at the time) was also a

cause of human infections came in 1990 with a report of cases of GAE due to leptomyxid; the first U.S. was later retrospectively identified as occurring in 1978.[16]

N. fowleri primarily affects immunocompetent children and young adults and without treatment has a rapid clinical course resulting in death within 1 to 2 weeks after exposure.[1] In 1980, Martinez was the first to note that *Acanthamoeba* is an opportunistic infection affecting persons with chronic illness.[17] This characterization seems to remain true as most reviews consider it to be an infection that typically affects immunocompromised persons.[1,2,17] It is thought that there is a subacute phase of infection and the timeframe has not been well characterized, but once the acute phase commences, the infection can be fatal within weeks. *B. mandrillaris* has been reported to affect both immuncompromised and healthy individuals.[1] Of note, recent reports of *Balamuthia* infection via organ transplantation raise concerns for the organ procurement community.[18,19] The clinical course of balamuthiasis is thought to be the same as that of acanthamoebiasis. Like *Acanthamoeba*, *Balamuthia* can also infect tissues outside the central nervous system.[1] Unlike with *Acanthamoeba* and *Naegleria*, it has been reported that being of Hispanic ethnicity may be a risk factor for *B. mandrillaris* infection.[1,20]

There are subtle differences in the routes of human infection among the free-living amebae. *N. fowleri* enter the nasal cavity, attach to the nasal mucosa, cross the nasal-brain (cribriform) barrier, and invade the brain. This invasion results in hemorrhagic necrosis as the amebae lyse or ingest red blood cells and/or digest or destroy brain tissue through secretions in their food cups.[1,11] *Acanthamoeba* spp. is thought to invade the body via the nasopharyngeal route, breaks in the skin, or by inhalation of cysts. The organism also produces food cups and secretes enzymes that may aid its

spread throughout the body. *Balamuthia* is thought to have some similarity to *Acanthamoeba* in its route of infection and its possession of particular enzymes that aid in its invasion process, but little is known about its pathogenesis.[1]

Meningoencephalitis is the swelling of the brain and its membranes. Butt first introduced the concept of primary amebic infection to differentiate between *Entamoeba histolytica*'s secondary infection of the brain (via the bloodstream) and *Naegleria*'s direct infection.[11] Later, Martinez differentiated between the manifestations of free-living amebae infections caused by *Acanthamoeba* and *Naegleria*, particularly the changes occurring in the tissues of the brain, giving further distinguishing differences between PAM and GAE. PAM was characterized as "severe acute necrotizing and hemorrhagic meningoencephalitis involving mainly the base of frontal lobes and posterior fossa" (p. 570) with lesions located on the surface of the brainstem, cerebellum, and cerebral hemispheres.[17] GAE's characteristics included: leptomeninges with moderate amount of purulent exudate over most affected cortical areas, moderate to severe edema in the cerebral hemisphere, and "foci of encephalomalacic worsening associated with small areas of hemorrhagic necrosis in occipital, parietal, temporal, or (less often) frontal lobes" (p. 569). GAE lesions vary in their location and composition.[17]

Purpose of the Study

There have been many case reports of free-living ameba infections in the United States, publications on diagnostic techniques and immunology, and reviews of the organisms; but there is still much to learn about the epidemiology and clinical course of these infections. A few case reports and reviews have attempted to summarize a limited number of published cases of acanthamoebiasis and balamuthiasis,[21,22] or have examined

specific aspects of these infections, such as case reports of *Acanthamoeba* GAE or *Acanthamoeba* rhinosinusitis among AIDS patients.[23,24] Only two papers have examined data from the literature and cases reported to CDC, and both were published over twenty years ago.[6,16] A recently published article concerning the epidemiology of primary amebic meningoencephalitis caused by *Naegleria fowleri* in the United States gives a comprehensive review of cases using multiple sources of information such as the Centers for Disease Control and Prevention's (CDC) Free-Living Ameba (FLA) Laboratory case registry and Waterborne Disease and Outbreak Surveillance System, as well as the National Vital Statistics System, published case reports, and media reports.[25] The descriptive analysis presented within this thesis is modeled on the methodology of the PAM epidemiology paper and will examine *Balamuthia* and non-keratitis *Acanthamoeba* cases in the United States from 1955 to 2009 by accessing the FLA Laboratory case registry and published case and media reports.

The purpose of this study is to better document the epidemiology of *Balamuthia* and non-keratitis *Acanthamoeba*, give insight into trends of these infections over time, and contribute to the scientific and medical community by producing the only comprehensive review of all known U.S. cases of *Balamuthia* and non-keratitis *Acanthamoeba* through 2009. Along with bringing about a better understanding of the characteristics of these cases, this study will also examine cases that have survived and attempt to determine if there is any evidence for a given treatment regimen as being particularly effective. Only a small number of patients have survived these infections, and any evidence for a successful course of treatment could be crucial for future cases.

Research questions

1. Are there any demographic characteristics that indicate commonality between cases and suggest that persons of a particular ethnicity or race are more pre-disposed to infection? In particular, does *Balamuthia* disproportionately affect persons of Hispanic ethnicity as suggested in the literature?
2. Are there any presenting symptoms that are particular to acanthamoebiasis and balamuthiasis that would assist clinicians in early identification of free-living ameba infection?
3. Are there any commonalities in the treatment regimen of those who survived?
4. What is the time from onset of symptoms to death?

CHAPTER II: REVIEW OF THE LITERATURE

There have been more than 60 case reviews or reports citing cases of non-keratitis *Acanthamoeba* disease and over 40 cases of *Balamuthia* disease in the United States published between 1960 and 2009. The first case report of *Acanthamoeba* disease was published in 1960, five years after the actual case occurred, and was reported as a granuloma of the brain, likely due *Endolimax williamsi*.[26] This same case was cited in several subsequent publications as *Acanthamoeba* granulomatous amebic encephalitis (GAE) upon retrospective review.[6,17,21,27] The first case report of *Balamuthia* disease was published in 1974; although *Balamuthia* was not recognized as the causal agent at the time, it was reported as a case of free-living amebic meningoencephalitis that was not due to *Naegleria* or *Acanthamoeba*.[28] The case was cited as *Balamuthia* GAE in subsequent publications.[6,17,27] Appendix A has detailed tables of all published *Acanthamoeba* and *Balamuthia* case reports.

Disseminated infections

A review of the literature reveals *Acanthamoeba* disease manifests itself in various ways (disseminated, GAE, cutaneous, rhinosinusitis, osteomyelitis) as does *Balamuthia* (disseminated and GAE). But unlike *Acanthamoeba*, disseminated balamuthiasis always involves the brain. Disseminated *Acanthamoeba* disease was the most frequently reported manifestation of acanthamoebiasis in the United States, with 29 cases published in the literature. [See Appendix A, Table 1 for all disseminated acanthamoebiasis case references.] The majority (n=19) of reported disseminated

infections were in patients who had human immunodeficiency virus (HIV) or who had acquired immune deficiency syndrome (AIDS). [See cases 5-11, 13-19, 21, 22, 24, 25, and 28.] While HIV/AIDS was the predominant cause for immunosuppression among case patients with disseminated acanthamoebiasis reported in the literature, it was followed by those with a previous medical history of organ transplantation and immunosuppressive drug therapy (n=4; cases 4, 23, 27, and 29) and bone marrow transplants due to cancer (n=3; cases 12, 20, 26). One case was reported as having lupus and two case reports cited no known predisposing health factors (cases 3, 1, and 2 respectively).

The most common form of disseminated *Acanthamoeba* disease was a combination of skin and sinus infection (n=10; cases 5, 8, 11, 13-15, 21, 22, 24, 28), followed by cases with a combination of GAE and cutaneous infection (n=3; cases 6, 9, 20). There were two reports of patients with GAE, cutaneous, and lung disease (cases 3, 29), two reports of patients with GAE and lung disease (cases 10, 26), and two cases with cutaneous infection and osteomyelitis (cases 17, 27). The other disseminated manifestations varied greatly among the patients. Those cases that included GAE and other manifestations were: GAE, cutaneous, and sinus (n=1; case 19); GAE and keratitis (n=1; case 1); GAE, lungs, and adrenals (n=1; case 12); GAE, cutaneous, and pulmonary hyaline membrane (n=1; case 4); and GAE, cutaneous, sinus, lymph nodes, thyroid, and adrenals (n=1; case 2). Other disseminated cases included: cutaneous, lungs, and keratitis (n=1; case 7); cutaneous, lungs, heart, kidney, spleen, and lymph nodes (n=1; case 16); cutaneous, sinus, and osteomyelitis (n=1; case 18); cutaneous, liver, and bronchoaveolar lavage (n=1; case 23). One case reported sinus and lung involvement (case 25).

The majority of the disseminated acanthamoebiasis cases were in adult males (n=18; cases 4-10, 13-15, 18, 19, 21, 22, 24-26, 29), and all children (<18 years of age) reported to have disseminated acanthamoebiasis were boys (n=4; cases 1, 11, 16, 17). Seven females were reported with disseminated disease (cases 2, 3, 12, 20, 23, 27, 28). Three reports noted that the patients were of Hispanic ethnicity (cases 2, 11, and 14) although two additional cases were perhaps of Hispanic ethnicity (cases 6 and 28), being described as Mexican American and Nicaraguan, respectively. However, the other 26 case reports failed to describe ethnicity, so an assessment of proportion of cases by ethnicity cannot be made. Only two reports included information about possible environmental exposures and they were both water exposures, one from fishing on a freshwater lake (case 29) and one from wading in drainage ditches (case 1). When timeframes were able to be determined, the number of days between suspected onset of symptoms and death ranged from 8 to 765 days (cases 1-3, 5, 6, 8, 10-13, 16, 19-23, 25, 26, 29). Three cases resolved their infections and did not die (cases 17, 23, 24) and eight did not have adequate information on the symptom onset/death timeframe to determine outcome status (cases 4, 7, 9, 14, 15, 18, 27, 28). The number of days between hospitalization and death ranged from 2 to 120 days (cases 1-7, 9, 10, 14, 15, 18, 25, 26, 27, 28). Twelve cases did not have adequate information on the hospitalization/death timeframe (cases 7, 8, 11, 12, 13, 16, 17, 19, 20, 21, 22, 28). Prognosis for those diagnosed with disseminated disease was poor, with 25 cases having died either from *Acanthamoeba* infection or from other complications (cases 1-16, 18-21, 25-29). In one case, it is unknown whether the patient survived (cases 22). Three cases were still living at the time of the published report, with follow-ups of one month (case 24) and 12

months (case 23). The third report did not give a timeframe but mentioned the patient was still being maintained on therapy (case 17).

In contrast, only seven cases of disseminated *Balamuthia* have been reported. [See Appendix A, Table 2 for all disseminated balamuthiasis case references.] The information concerning potential health-related predisposing factors for these cases was limited and varied, including a history of alcohol or drug abuse (n=3; cases 1, 2, 6), otitis media (n=1, case 5), a history of a farm accident resulting in a contaminated wound and subsequent limb amputation (n=1, case 3), and ankylosing spondylitis (n=1; case 4). One case-patient had a history of coronary artery disease (case 7), but it is unclear whether this medical condition was significant or not given that *Balamuthia* organisms were found in the CSF and skin but nowhere else. Unlike acanthamoebiasis, all the manifestations of disseminated balamuthiasis included GAE. The majority of cases were GAE and cutaneous infections (n=4; cases 3, 4, 6, 7), and the rest were GAE with infection of another organ outside the brain (n=3; cases 1, 2, 5).

As with disseminated acanthamoebiasis, the majority of cases of disseminated balamuthiasis were in adult males (n=6; cases 1-4, 6, 7). Only one child was reported to have this manifestation of illness and this case was female (case 5); this same case was reported as being of Hispanic ethnicity and having a travel history to Mexico. Ethnicity was reported in one other case (case 6, Hispanic), but the remaining 5 case-patients with disseminated balamuthiasis had no ethnicity reported so no pattern can be determined from these data. Environmental exposure history was limited with only two reports providing information: both mentioned potential soil exposures (case 3, 4). When timeframes could be determined, the number of days between suspected onset of

symptoms and death ranged from 24 to 568 days (cases 1-3, 5-7). One case-patient resolved his infection and did not die, but the case report did not provide adequate information to determine the time period between symptom onset and resolution (case 4). The number of days between hospitalization and death ranged from 13 to 77 days (cases 1-3, 5-7). Clinical outcomes for disseminated *Balamuthia* disease were also poor, with six of the case-patients dying (cases 1-3, 5-7) and one reported survivor (case 4). This patient was followed for more than five years and seemed to have recovered from his infection.

Granulomatous amebic encephalitis (GAE)

There are 21 published reports of granulomatous amebic encephalitis caused by *Acanthamoeba* spp. from 1955 to 2009. [See Appendix A, Table 3 for all *Acanthamoeba* GAE case references.] Similar to the disseminated form of acanthamoebiasis, the most common predisposing condition among infected patients was HIV/AIDS (n=4, cases 10, 11, 14, 20), and an equal number of patients with GAE had a history of substance and alcohol abuse (n=4; cases 2, 3, 15, 21). Other potential predisposing health conditions included: cancer (n=2) with long-term immunosuppression (case 5) and with a stem cell transplant (case 16); organ transplantation (n=2) with immunosuppressive therapy (cases 17) and with diabetes (case 19); lupus (n=2; case 18), where one case-patient was also dependent on drugs and alcohol and counted above as having history of substance abuse (case 21); mixed connective tissue disorder (n=1; case 9); and pneumonitis (n=1, case 6). One case-patient (case 4) had a stroke, but it is unknown if this may be a predisposing condition. Of the 21 reports, five had either unknown or unpublished health conditions (cases 1, 7, 8, 12, 13) that may have made them more susceptible to infection.

The majority of cases of *Acanthamoeba* GAE were adult males (n=13; cases 2-5, 10-15, 17, 19, 20). Only three cases were in children under the age of 18, and all of them were female (cases 1, 7, 8). There were five adult females with *Acanthamoeba* GAE (cases 6, 9, 16, 18, 21). No case reports noted patients of Hispanic ethnicity. Two reports noted that case-patients had water exposures; one case-patient was exposed to water that had accumulated in a basement (case 3), but the source of the water was not specified, and the other case-patient frequently used swimming pools for therapy after a cerebrovascular accident (case 4). A third case-patient, a male, had multiple exposures, including a potential occupational exposure (general contractor) and exposures to water and soil. He played volleyball on sand courts and also gardened as a hobby. He also enjoyed fishing and was on a trip approximately one month prior to his illness onset, although he denied direct water contact (case 19). The remaining 18 case reports did not comment on potential environmental exposures. When timeframes could be determined, the number of days between suspected onset of symptoms and death ranged from 8 to 252 days (cases 1, 5, 6, 9, 10, 11, 14-21). Seven did not have adequate information on the symptom onset/death timeframe (cases 2-4, 7, 8, 12, 13). The number of days between hospitalization and death ranged anywhere from 4 to 63 days (cases 1, 2, 3, 5, 9, 10, 11, 14, 16, 17, 21). Ten cases did not have adequate information on the hospitalization/death timeframe (cases 4, 6-8, 12, 13, 15, 19, 20). Among cases where the clinical outcome was known, fatality was 100% (cases 1-7, 9-11, 14-21). Three cases had unknown outcomes (cases 8, 12, 13).

Thirty-nine cases of *Balamuthia* GAE in the United States have been published in case reports or cited in summary compilations. [See Appendix A, Table 4 for all

Balamuthia GAE case references.] Eleven case reports did not include information that lent evidence of predisposing health factors, nor was the patient's status stated as being healthy (cases 3, 4, 6, 9, 23-25, 30, 31, 35, 36). Nine cases were reported as being previously healthy before becoming ill (cases 7, 10, 12, 13, 15, 20, 21, 27, 32). In instances where predisposing health factors were known, nine cases had otitis, pharyngitis, or some other type of respiratory illness (cases 2, 5, 11, 14, 18, 19, 22, 33, 37). Five cases had a history of alcohol or drug abuse (cases 5 [also had TB as noted above], 8, 17, 26, 29,). Other predisposing health factors included: organ transplantation and immunosuppressive therapy (cases 34, 38, 39), HIV (case 16), prolonged steroid use (case 28), and diabetes (case 1).

Unlike *Acanthamoeba* GAE, the majority of cases of *Balamuthia* GAE were in males younger than the age of 18 years (n= 14; cases 2, 3, 9, 13-15, 18, 23, 24, 28, 30, 32, 36, 37) followed by adult males (n= 11; cases 5, 6, 8, 17, 25, 26, 29, 31, 33, 35, 39). There were eight case reports on females under age 18 (cases 4, 7, 10-12, 20-22) and five adult women (cases 1, 16, 19, 27, 38). One case report did not include any age or gender information (case 34). Fourteen of the cases (cases 7, 10, 15, 17, 21-25, 28-31, 36) were reported to be of Hispanic ethnicity; the rest of the case reports did not include information on ethnicity. Environmental exposure to soil was cited in six cases (cases 7, 25, 27, 30, 33, 35). Two patients cited gardening as a hobby (cases 27, 35), two had an occupational exposure to soil (case 25, 33), one cited motorcycling in the desert as a hobby (case 30), and one visited a farm (case 7). One case-patient had a history of frequent outdoor play in soil as well as exposure to an untreated well-water source for recreation and drinking (case 37). Three additional cases cited water exposure, one to a

freshwater pond (case 20), one to well water (case 1), and one to a lake (the latter may have also had soil exposure as the child of a migrant farmer, case 9). When timeframes were able to be determined, the number of days between suspected onset of symptoms and death ranged from 8 to 240 days (cases 1-5, 7, 10, 12-15, 17-19, 22, 24, 25, 33, 37, 38). Five cases resolved their infections and did not die (cases 21, 27, 31, 32, 39) and fourteen did not have adequate information on the symptom onset/death timeframe (cases 6, 8, 9, 11, 16, 20, 23, 26, 28, 29, 30, 34, 35, 36). The number of days between hospitalization and death ranged anywhere from 3 to 120 days (cases 1, 2, 9, 11, 16, 20, 26, 28-30, 35, 36). Twenty-two cases did not have adequate information on the hospitalization/death timeframe (cases 3-8, 10, 12-15, 17-19, 22-25, 33, 34, 37, 38). Survival for those with *Balamuthia* GAE was slightly better for those with GAE caused by *Acanthamoeba* spp. Four patients are reported to have survived (case 21, 27, 31, 39) and the other 35 died (cases 1-20, 22-26, 28-30, 32-38).

Cutaneous acanthamoebiasis

Twelve cases of cutaneous acathamoebiasis occurring in the United States have been reported in the literature and all cases were among immunocompromised adults. [See Appendix A, Table 5 for all cutaneous acanthamoebiasis case references.] The main predisposing medical condition was HIV/AIDS (n=10; cases 1-4, 6-11). The only other condition cited in the literature was immunosuppression due to organ transplantation (n=2; cases 5, 12). Nine of the case-patients were males (cases 1-7, 9, 10) and three were females (cases 8, 11, 12). Two case reports noted that the patients were of Hispanic ethnicity (cases 2, 8), but ethnicities were not reported for the other 10 case-patients. No soil exposures were cited in the case reports, although two reports noted cases had

freshwater exposure before skin lesions appeared (cases 6, 7). One case was noted to have a local creek nearby that was used as a domestic drinking water source (case 12). No potential environmental exposures were cited for the remaining nine case-patients. When timeframes could be determined, the number of days between suspected onset of symptoms and death ranged from 22 to 450 days (cases 1-4, 6, 8-11). Two cases resolved their infections and did not die (cases 5, 12) but the symptom onset/death timeframes could not be determined and one fatal case did not have adequate information on the symptom onset/death timeframe (case 7). The only report of number of days hospitalized was in a survivor who was hospitalized for approximately 150 days (case 12). The remaining cases did not have adequate information on the hospitalization/death or hospitalization/discharge timeframes (cases 1-11). There were three reported survivors of cutaneous *Acanthamoeba* (cases 5, 11, 12) and the other nine cases resulted in death (cases 1-4, 6-11).

Acanthamoeba rhinosinusitis

Two cases of *Acanthamoeba* rhinosinusitis have been reported in the literature. [See Appendix A, Table 6 for all *Acanthamoeba* rhinosinusitis case references.] Both patients were immunocompromised adults, one male (case 1) and one female (case 2). The male had HIV and recurrent sinus problems and the female had undergone a bilateral lung transplantation due to a progressive lung disease. Both of these patients are reported to have resolved their infections at 4 (case 1) and 3 (case 2) weeks respectively, but no further follow-up was mentioned in either article.

Acanthamoeba osteomyelitis

A lone case of *Acanthamoeba* osteomyelitis was reported in 1981 in a 32-year-old

pre-diabetic woman. [See Appendix A, Table 7 for all *Acanthamoeba* osteomyelitis case references.] She had a mass in the right mandibular area, which was removed and replaced with a bone graft. A subsequent surgery was necessary in order to remove necrotic bone. Inflammatory exudate from bone marrow in the necrotic bone was positive for *Acanthamoeba*. The report does not cite any follow-up after the patient's surgery for necrotic bone removal, so it is not known if she continued to improve and remain ameba-free (case 1). Three other cases of *Acanthamoeba* osteomyelitis have been reported in the literature, but all of these case patients had disseminated *Acanthamoeba* infection (see Appendix A, Table 1, cases 17, 18, 27).

Survivors: Acanthamoeba

There have been nine reports of successful treatment of acanthamoebiasis: three disseminated (Appendix A, Table 1, cases 17, 23, 24), three cutaneous (Appendix A; Table 4, cases 5, 11, 12), two rhinosinusitis (Appendix A, Table 5, cases 1, 2), and one osteomyelitis (Appendix A, Table 6, case 1). There have been four published cases where the clinical outcome was unknown due to lack of information in the reports: one was a disseminated disease case (Appendix A, Table 1, case 22) and three were GAE cases (Appendix A, Table 3, cases 8, 12, 13). In 1998, a report was published of a 7-year-old male with HIV who had been diagnosed with cutaneous *Acanthamoeba* disease at 5 ½ years of age (Appendix A, Table 1, case 17). Initially this case-patient was treated with fluconazole without complete resolution of his skin lesions. Several months after his diagnosis of cutaneous *Acanthamoeba* disease, he had a painful thumb and testing showed osteomyelitis without a definitive cause. He was given pentamidine daily over the course of a month and then maintained on itraconzole with monthly treatments of

pentamidine. By the time he was 6-years-old, he was diagnosed with granulomatous amebic osteomyelitis after presenting with a swollen elbow. He was again given pentamidine daily for one month. His cutaneous lesions and osteomyelitis recurred when he was close to 7 years old, despite maintenance therapy. Daily pentamidine for one month was given in combination with 5-fluorocytosine (5-FC). The child was then maintained on 5-FC and antiretroviral treatment and was alive at the time of publication but had occasional new skin lesions.

In 1999 a report was published of a 39-year-old woman who had undergone a lung transplant six years earlier (Appendix A, Table 1, case 23). She was reported to have presented with skin nodules, with new nodules continuing to develop over the next several days. Multiple biopsies were performed before a diagnosis of *Acanthamoeba* was finally made. Initial treatment included a combination of 5-FC and pentamidine, as well as topical treatment with chlorhexidine gluconate and ketoconazole cream. Upon going into respiratory failure and requiring mechanical ventilation, the patient's bronchoalveolar lavage (BAL) was found to be positive for *Acanthamoeba*. At this point azithromycin was added to her drug regimen and it appears she stayed on this regimen for close to a month, at which time her skin lesions were improving and her BAL was negative. However, she began to have side effects that resulted in several of the medications being discontinued. The patient had abnormal liver function tests and a biopsy showed *Acanthamoeba* was present in this organ, so the patient was given lower doses of pentamidine and 5-FC was restarted. On discharge, the patient was still being treated with 5-FC and clarithromycin was added. The woman showed no evidence of infection 12 months after her hospital discharge.

In 2002, a 37-year-old AIDS and cancer patient was reported to have nasal obstruction that did not resolve with antibiotic treatment (Appendix A, Table 1, case 24). He subsequently developed papular lesions on his extremities. Multiple biopsies were inconclusive, although Cytomeglovirus was suspected and treatment was begun; the patient did not respond well. Subsequently, the nasal obstruction became worse until the patient needed surgery, at which point a diagnosis of *Acanthamoeba* was made from the specimens obtained during surgery. The patient was given pentamidine and topical treatments of both chlorhexidine and ketoconazole. When he had side effects due to pentamidine it was discontinued and 5-FC was begun; this regimen was continued for a month with successful resolution of his disease. At the time of the report, the patient was still taking itraconazole but there was no evidence of recurrence. The report does not mention when treatment with this drug was begun.

Three cases of cutaneous acathamoebiasis were reported to have survived. In 1992, a 31-year-old man who had undergone a kidney transplant presented with skin nodules (Appendix A, Table 5, case 5). Upon diagnosis of *Acathamoeba* via positive skin biopsies, he was given intravenous (IV) pentamidine and topical treatment with chlorhexidine and ketoconzaole. He was treated with pentamidine for 4 weeks, although the dosage had to be reduced due to an elevated creatinine level. At discharge from hospital, his previous medications were discontinued and he was then treated with oral itraconazole. He continued to take this medicine at a reduced dosage for at least two years after his diagnosis without recurrence of infection.

In 2004, a 51-year-old woman with AIDS presented with skin papules, photophobia, and headache (Appendix A, Table 5, case 11). Based on a skin biopsy, she

was diagnosed with cutaneous *Acanthamoeba* disease and underwent a CT scan and lumbar puncture, which were subsequently negative for evidence of central nervous system involvement. Her treatment regimen included pentamidine and 5-FC, but new skin nodules continued to appear so sulfadiazine and highly active antiretroviral treatment (HAART) were added. After 14 days of pentamidine once a day, penatmidine was discontinued and the patient was continued on maintenance therapy of 5-FC, sulfadiazine, and HAART. The patient was still free of CNS involvement 3 months after release from the hospital with slow improvement of her skin lesions, possibly due to inconsistent compliance with her treatment.

Also in 2004, a 52-year-old woman who had a lung transplant three years earlier and remained on immunosuppressive therapy presented with nodules on her trunk and lower extremities (Appendix A, Table 5, case 12). She was diagnosed with cutaneous *Acanthamoeba* disease and unsuccessfully treated with itraconazole and metronidazole for two weeks. CDC and an infectious disease specialist were consulted and the woman was started on IV amphotericin B lipid complex and voriconazole. She was discharged on the same medications delivered through an outpatient service. Her treatment with these two drugs lasted 10 weeks before the dosage and frequency were changed and IV voriconzole was switched to oral voriconazole. After her discharge she was maintained on oral voriconazole for 5 months with resolution of her skin lesions and no recurrence.

Two cases of *Acanthamoeba* rhinosinusitis were reported to have survived their infections. A 45-year-old man with HIV had sinus difficulties over the course of a year which necessitated multiple sinus surgeries before he was finally diagnosed with *Acanthamoeba* rhinosinusitis (Appendix A, Table 6, case 1). He had a subtotal

septectomy, used gentamycin nasal rinses for 4 weeks and was maintained on oral itraconazole. No timeframe was given for the maintenance therapy, nor was the time frame between the resolution of the infection and the publication of the paper given. In 2005, a 49-year-old woman who had undergone a lung transplant was reported to have survived *Acanthamoeba* rhinosinusitis (Appendix A, Table 6, case 2). Seven months after transplantation she had sinus difficulties and after diagnosis was treated with amphotericin, voriconazole, and caspofungin. Three weeks after treatment began she underwent a sinus debridement, but no *Acanthamoeba* were seen in specimens from this debridement: she remained *Acanthamoeba* free in the month following which is as long as the report states the patient was followed.

The only other published report of an *Acanthamoeba* survivor was in 1981 (Appendix A, Table 7, case 1). A 32-year-old female was diagnosed with amebic osteomyelitis after a sequestrum of necrotic bone was removed from her jaw. The patient does not appear to have been treated with drugs that were specific to resolving *Acanthamoeba* infections. She was given IV penicillin, used betadine mouthwash after surgery, and was discharged. The authors do not discuss long-term follow up, but as she was reported as still alive at the time of the published report she is counted among the survivors in this review. As with many of these cases where patients were reported to have survived, it is difficult to determine if there were later recurrences or if patients truly resolved their infections and never relapsed.

Survivors: Balamuthia

There have been six reports of successful treatment of balamuthiasis, one with disseminated disease (Appendix A, Table 2, case 4) and five with *Balamuthia* GAE

(Appendix A, Table 4, cases 21, 27, 31, 32, 39). A 64-year-old man who previously had a skin lesion that was biopsied with pending results presented with neurological difficulties (Appendix A, Table 2, case 4). Lesions were seen in the parietal area of his brain on both a CT scan and a MRI. He subsequently underwent a brain biopsy and was released 10 days later. He was then re-hospitalized and received a diagnosis of *B. mandrillaris* disseminated disease as *Balamuthia* organisms were identified in both the brain tissue and the skin biopsy. He was treated with 5-FC, fluconazole, IV pentamidine, sulfadiazine, and azithromycin (which was later switched to clarithromycin). The patient was ventilated and in intensive care for seven weeks. He was discharged to a rehabilitation unit and fluconazole was discontinued. Following a relapse, fluconazole was added again and he was maintained on fluconazole and sulfadiazine over the course of five years. The patient did recover enough to be able to walk, perform activities of daily living and communicate well.

There have been five reports of survivors who were diagnosed with *Balamuthia* GAE. Three of the case-patients were reported as previously healthy (Appendix A, Table 4, cases 21, 27, 32) and one had an unknown health status (Appendix A, Table 4, case 31). A report published in 2003 discusses a 5-year-old girl who, 48 days after initial presentation, was diagnosed with *Balamuthia* GAE (Appendix A, Table 4, case 21). The patient underwent several combinations of treatment. Initially she was treated with ketoconazole and metronidazole for 34 days. These medications were then discontinued and she was placed on clarithromycin and flucytosine for 14 days. Her treatment was subsequently changed again and included azithromycin, fluconazole, pentamidine, and thioridazine. Over the course of the next two-and-a-half years, her treatment varied,

primarily because of discontinuation of medications due to side effects and initiation of medicines due to seizures. More than two-and-a-half years after her first hospital admission she was still maintained on clarithromycin and fluconazole.

In 2004, a report was published of a 72-year-old woman who underwent a brain biopsy that was positive for *Balamuthia* (Appendix A, Table 4, case 27). The biopsy was excisional, and upon diagnosis, the patient was treated with pentamidine (IV), sulfadiazine, fluconazole, and clarithromycin. The patient remained asymptomatic, but it is unknown how much time elapsed between the case and the published report as well as how long she was maintained on therapy. In 2007 a *Balamuthia* GAE survivor was reported (Appendix A, Table 4, case 31). The report only stated that the patient was living at the time of his last report to a physician, but he was lost to follow-up.

In 2010, a report of a 2-year-old boy with GAE was published (Appendix A, Table 4, case 32). He was initially treated for tuberculosis but underwent a biopsy and was given a diagnosis of *Balamuthia*. He was treated with pentamidine, fluconazole, flucytosine, sulfadiazine, clarithromycin, and thioridazine. The boy was placed on a ventilator and required a ventriculoperitoneal shunt. He was maintained on a ventilator for almost two months and then transferred to a rehabiliation unit. Over the course of at least 22 months, the case-patient was given clarithromycin, sulfadiazine, flucytosine, and fluconazole without evidence of an active infection. After time in the rehabilitation unit, the boy was able to follow simple commands, had improved postural stability, and was attempting to speak. MRIs show that the lesions decreased in size and number.

The most recent report of a *Balamuthia* GAE survivor was published in 2010 (Appendix A, Table 4, case 39). A kidney transplant recipient who acquired his infection

through organ transplantation from an infected donor survived the infection, although he spent two months in a coma. He slowly regained cognitive and motor functions and entered a rehabilitation unit. He had residual neurologic effects such as right arm paralysis and bilateral leg weakness. He also had intermittent vision loss. No treatment regimen was included in the publication.

Ethnicity

In 2004, a letter to the editor of *Emerging Infectious Diseases* was published, suggesting that Hispanic ethnicity may be a risk factor for *Balamuthia* GAE.[20] The primary authors of this letter were involved with the California Encephalitis Project (CEP), which was begun in 1998 with the purpose of assisting clinicians in the State with cases of encephalitis by offering enhanced diagnostic testing for infectious agents. The CEP collects serum and other samples from patients in California that have presented with encephalitis, and a subset of these samples were pulled for *Balamuthia* screening. The criteria for screening included: case history that had clinical or laboratory information suggestive of *Balamuthia* GAE and either an outdoor occupational exposure or other outdoor recreational activity that might have lead to exposure to soil. Of 215 samples tested, three were positive for *Balamuthia*. Four additional samples, not a part of the CEP, were also tested and were found to be positive. All seven cases were healthy Hispanic Americans who died of GAE. The CEP contacted CDC to obtain information about their records of *Balamuthia* cases, and it was determined that based on either case report identification of ethnicity or a traditional Hispanic last name, about 50%, or 25, of the U.S. case patients with known or suspected ethnicity were Hispanic Americans. No information was given on the number of cases with truly unknown ethnicities, just that

there were approximately 50 U.S. cases at the time of publication. This same letter also stated that 36% of *Balamuthia* GAE cases occurred in Latin American countries.[20]

CHAPTER III: METHODS AND PROCEDURES

CDC's Free-Living Ameba (FLA) Laboratory collects information on cases of suspected *Balamuthia* and non-keratitis *Acanthamoeba* diseases as part of their contact with physicians and laboratorians who are seeking assistance with diagnosis. The head of the FLA Laboratory, Dr. Govinda Visvesvara, has been working with free-living amebae since 1964 and has been with the FLA Laboratory since 1974. He was the first scientist to isolate and subsequently name *Balamuthia mandrillaris* and is considered one of the world's FLA experts, with more than 250 publications on the subject. The FLA Laboratory is one of the only research laboratories in the United States performing reference diagnostic testing for free-living amebae, and the lab handles specimens tested through various methods such as staining of histopathologic slides, culture, and polymerase chain reaction (PCR). The FLA Laboratory is one of only two known labs currently performing an antibody test (indirect immunofluorescence or IIF) for the free-living amebae; the other laboratory is at the California Department of Health Services, which runs the California Encephalitis Project (CEP). These two labs have historically shared data. Therefore, the FLA Laboratory holds records for most of the non-*Acanthamoeba* keratitis and *Balamuthia mandrillaris* FLA cases diagnosed in the United States. It is for this reason that a review of FLA laboratory records supplemented with a review of the published literature should capture the majority of FLA cases diagnosed in the United States, even though diseases associated with the free-living amebae are not nationally notifiable.

Data for this study were abstracted onto case report forms from CDC laboratory

and clinical consultation files for positive cases of *Balamuthia* and non-keratitis *Acanthamoeba* that occurred in the United States. See Appendix B for a copy of the case report form. Data in the files ranged greatly in content from laboratory specimen submission forms with little information about the patient's clinical course to copies of extensive medical records and autopsies.

A literature review was also performed to collect all published cases and media reports of *Balamuthia* and non-keratitis *Acanthamoeba* disease occurring in the U.S. The published cases were then compared to the CDC-diagnosed cases to determine which ones referred to the same patients. This was done in three ways: matching similar patient clinical and laboratory data, matching authors with contact clinicians or laboratorians, and consultating with Dr. Govinda Visvesvara, the aforementioned head of the FLA Laboratory. Information from published case reports took precedence over CDC records when there was conflicting data, unless the publication conflicted with a final autopsy report in CDC files. CDC records took precedence over any information found in the general media. In instances where there was information on length of time but not specific dates for illness onset, hospitalization, and/or time from illness onset to death, dummy dates were entered for the purpose of calculating durations.

In instances where published cases could not be matched with CDC cases, the diagnostic methods for the published cases were reviewed. Cases tested by the following agencies/individuals were considered to be reputable cases based on their known diagnostic methods: the California Department of Health Services; Dr. Eddy Willaert, Institute of Tropical Medicine in Antwerp, Belgium; Dr. Julio Martinez, University of Pittsburgh; Dr. Bruce Torian, Idaho; Dr. Gregory Booten, Ohio State University. If it

was not apparent that a published case was in CDC records, or if one of the laboratories above was not cited, individual cases were discussed with Dr. Visvesvara to determine if he was familiar with the case. Diagnostic and clinical features of the published case were reviewed to determine if the case likely fit a diagnosis of acanthamoebiasis or balamuthiasis.

Each case-patient identified from these records and/or the literature review was assigned a unique identifier consisting of a prefix of AC for *Acanthamoeba* or BA for *Balamuthia*, followed by a two digit number representing the calendar year in which the case first presented with symptoms (when known) or was either first reported to CDC or published in the literature. A case number in sequential order from 001 to infinity was designated as the last three digits of the unique identifier. Case information was then entered into a limited-user database that serves as a resource for CDC clinical consultation and surveillance.

The database has 976 variables and contains both quantitative and qualitative data. A major issue with the database is that many of the variables are set up as true/false, complicating the calculation of denominators for certain areas of data. The database allows exportation of files in Excel format and splits the database information into eight separate files when exporting: demographics, exposure history, medical history, current illness, laboratory tests, histopathology, diagnostic imaging, and diagnostic and treatment outcomes. For the purposes of this study, the information was stripped of identifiers prior to analysis. The data were then analyzed using SPSS.

Exported data were split into separate files for *Acanthamoeba* and *Balamuthia*. Each of the eight database files for each organism were imported into SPSS, labeled and

assigned type, values, and measures. All eight files were also combined for each organism for instances where information in the separate databases needed to be examined together. Data were first explored by examination of frequencies, ranges and means, and cross tabulations. Research question 1 was answered using these methods, and frequencies, ranges, and means were used for research question 4. For research question 2, frequencies were also used, but because acanthamoebiasis has so many manifestations, it was useful to explore the data by disease manifestation. Therefore, case summaries were run using final diagnosis as the grouping variable and presenting symptoms in the variables column. Research question 3 was also examined using case summaries with the survived variable as the grouping variable and treatment drugs in the variables column. Medications given to the survivors were visualized using Excel spreadsheets to determine commonalities in the drugs used and duration. Although the methods used above answered the research questions, I also further analyzed some of the demographic, exposure history, previous medical history, and outcomes data to try to glean further information about these diseases and produced epidemologic curves for all reported cases of acanthamoebiasis and balamuthiasis in the United States through 2009.

CHAPTER IV: RESULTS

A total of 114 cases of acanthamoebiasis was analyzed; 65 (57%) of these cases had some information about them published in the literature. Similarly, 70 cases of balamuthiasis were analyzed; 46 (66%) of these cases had some information about them published in the literature. Cases of acanthamoebiasis occurred between 1955 and 2009 (Figure 1); cases of balamuthiasis occurred between 1974 and 2009 (Figure 2). See Appendix C for larger versions of these figures.

Figure 1: Epidemic Curve for Cases of Non-Keratitis *Acanthamoeba* spp.—United States, 1955-2009

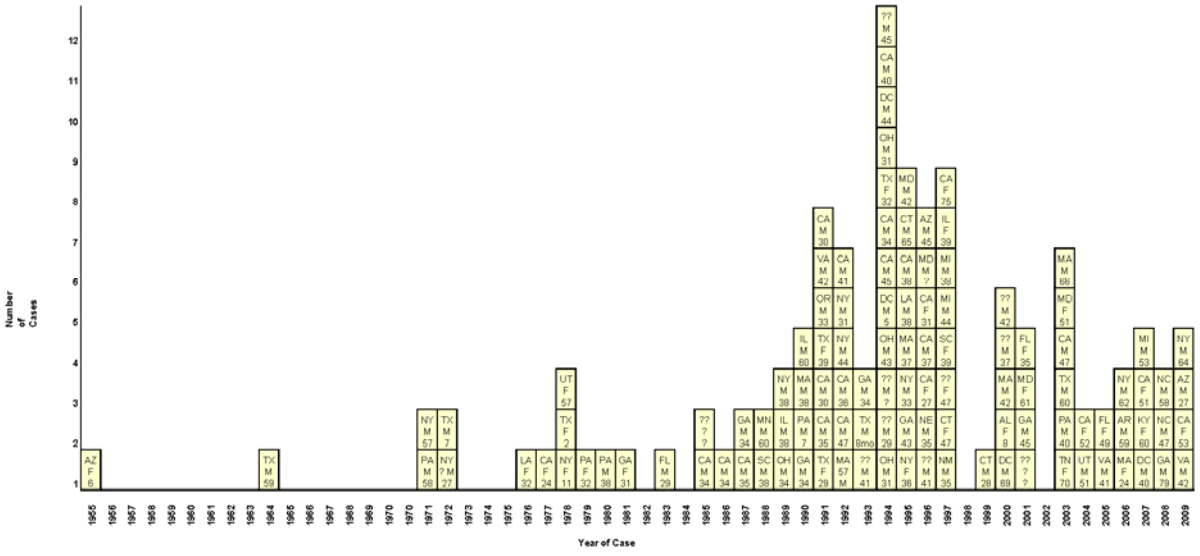
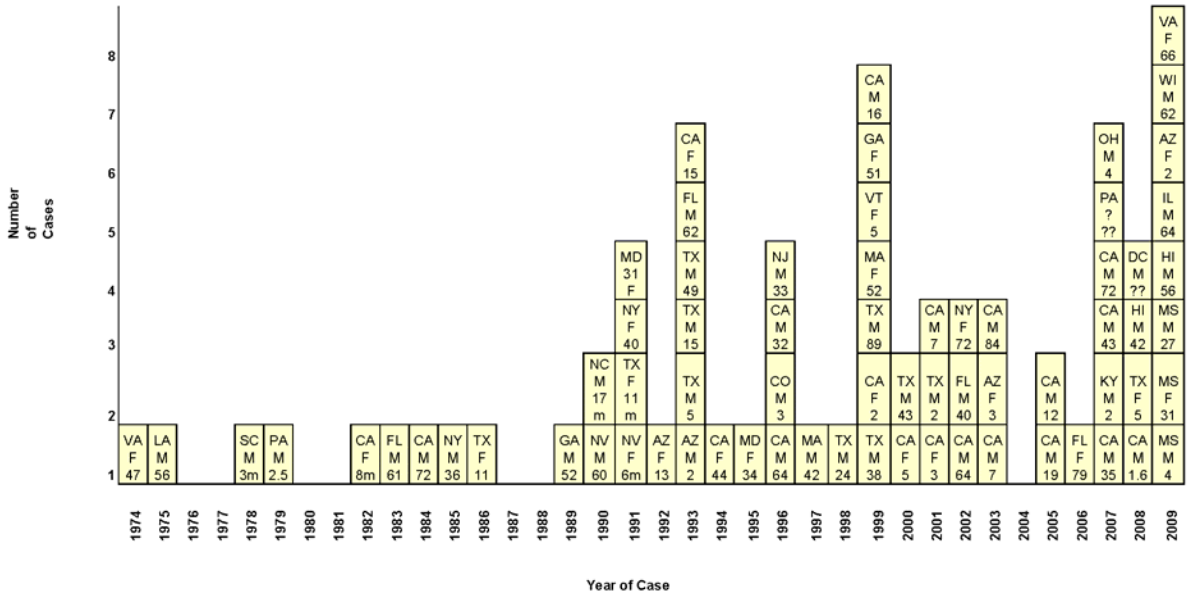


Figure 2: Epidemic Curve for Cases of *Balamuthia mandrillaris*—United States, 1974-2009



Demograhics: Research Question 1

Little information was available about the race and ethnicity of *Acanthamoeba* cases. Sixty-two percent of the race data were missing (n=43/114). Among the 43 case patients who had data available, the racial breakdown was: 63% white (n=27/43), 35% African American (n=15/43), and 2% American Indian (n=1/43). Ninety percent of the data were missing for the ethnicity variable (n=11/114), but among the known cases 82% (n=9/11) were Hispanic and 18% (n=2/11) were non-Hispanic. Five out of the nine known Hispanics were treated in the state of California. Immigration data were also limited; 93% of the data were missing (n=8/114), but when known 75% had immigrated to the United States (n=6/8) and 25% were natural U.S. residents (n=2/8). There was no particular country of emigration in common among those six cases who had immigrated. Four percent of cases were missing data for the age variable (n=110/114). Ages of cases ranged from a minimum of 8 months old to a maximum of 79 years old, with a mean of

38.8 years of age; 7% (n=8/110) were children younger than 18 years of age, 87% (n=96/110) were adults 18-64 years of age, and 6% (n=6/110) were 65 years of age or older. Males were 73% of cases (n=82/112) and females were 27% (n=30/112); two cases had missing information. See Figure 3 for an epidemic curve by age of *Acanthamoeba* spp. [Appendix C has a larger version of this figure.] Also see Figure 4 for the geographic distribution of *Acanthamoeba* spp. cases by state of treatment.

Figure 3: Epidemic Curve by Age for Cases of *Acanthamoeba* spp.—United States, 1955-2009

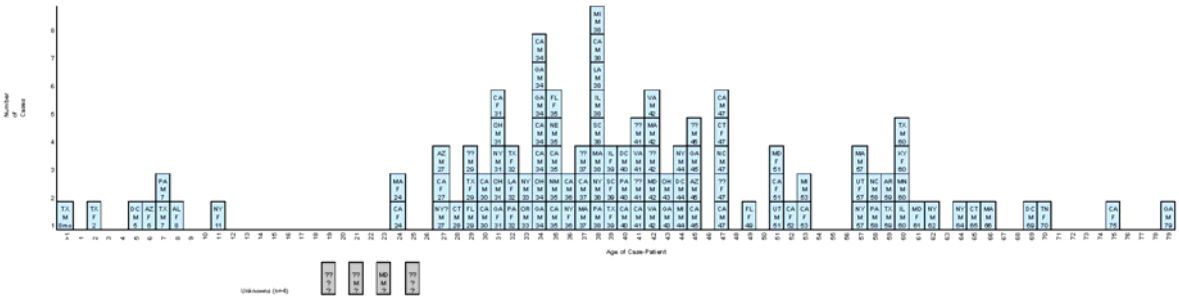
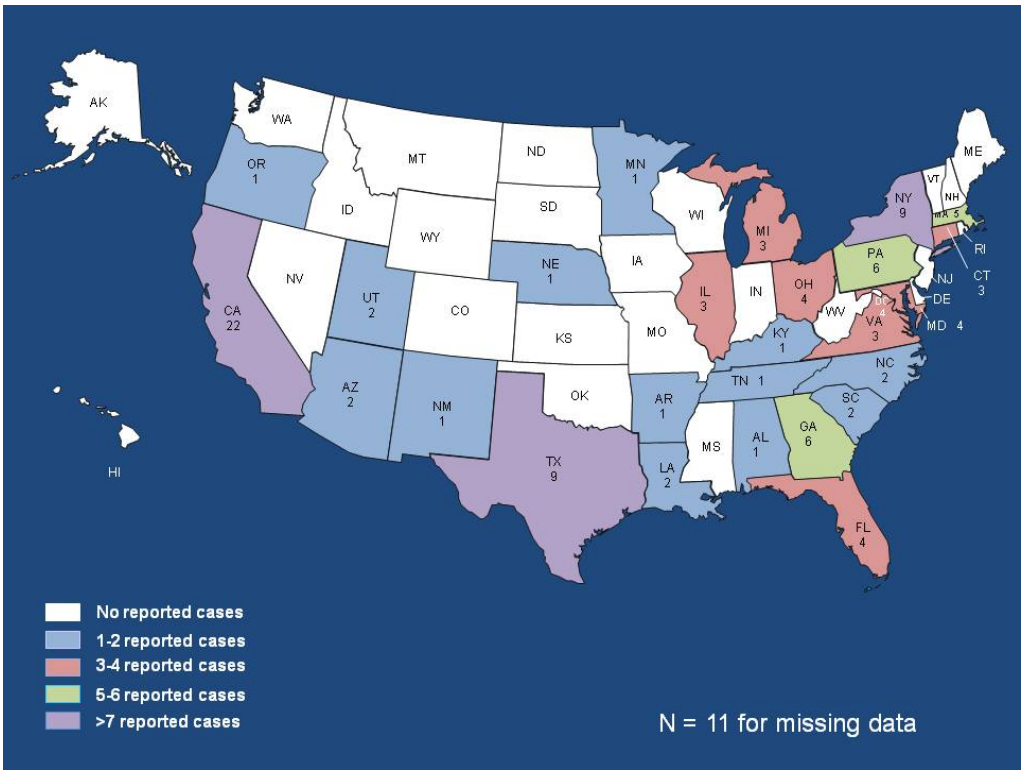


Figure 4: Geographic Distribution of Non-Keratitis *Acanthamoeba* spp. Cases by State of Treatment



Limited data were available for race, ethnicity, and immigration status for *Balamuthia* cases. Fifty-three percent of the race data were missing (n=33/70). Among cases where data were available, the racial breakdown was: 58% white (n=19/33), 36% African American (n=12/33), and 6% Asian/Pacific Islander (n=2/33). Fifty-nine percent of the data were missing for ethnicity (n=29/70), but among the known cases 76% were Hispanic (n=22/29) and 24% were non-Hispanic (n=7/29). Twelve of the 22 known Hispanic case-patients were treated in the state of California. The immigration variable had 84% of the data missing (n=11/70); 36% of case-patients with known immigrantion status immigrated to the U.S. (n=4/11) and 64% were natural residents of the U.S. (n=7/11). Immigrants most commonly came from Mexico (75%, n=3/4). Two cases were missing data for the age variable, with ages ranging from a minimum of 4 months to a maximum of 89 years of age, and a mean age of 30.4 years; 43% (n=29/68) were children younger than 18 years of age, 47% (n=32/68) were adults 18-64 years of age, and 10% (n=7/68) were 65 years of age or older. Males were 65% of cases (n=45/69) and females were 35% (n=24/69); only one case was missing gender information. See Figure 5 for an epidemic curve by age of *Acanthamoeba* spp. [Appendix C has a larger version of this figure.] Also see Figure 4 for the geographic distribution of *Balamuthia mandrillaris* cases by state of treatment.

Figure 5: Epidemic Curve by Age for Cases of *Balamuthia mandrillaris*—United States, 1974-2009

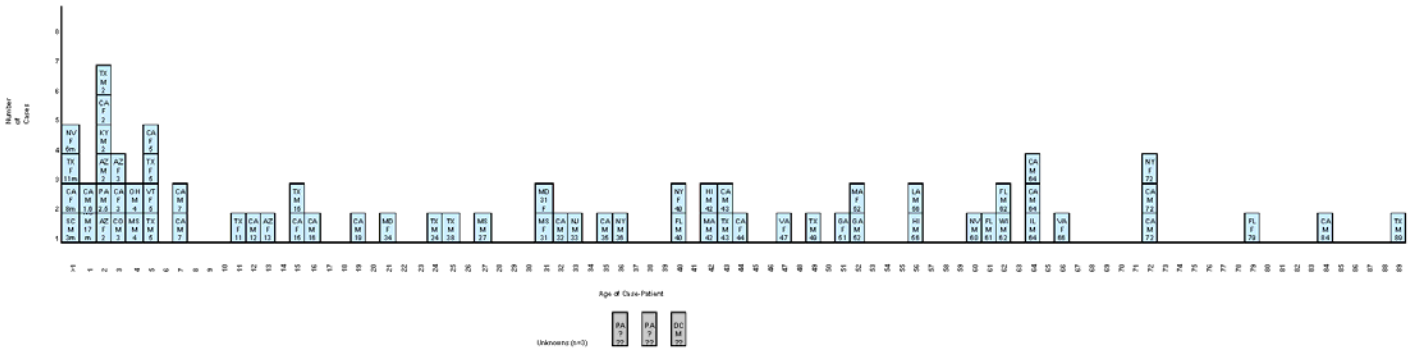


Figure 6: Geographic Distribution of *Balamuthia mandrillaris* Cases by State of Treatment

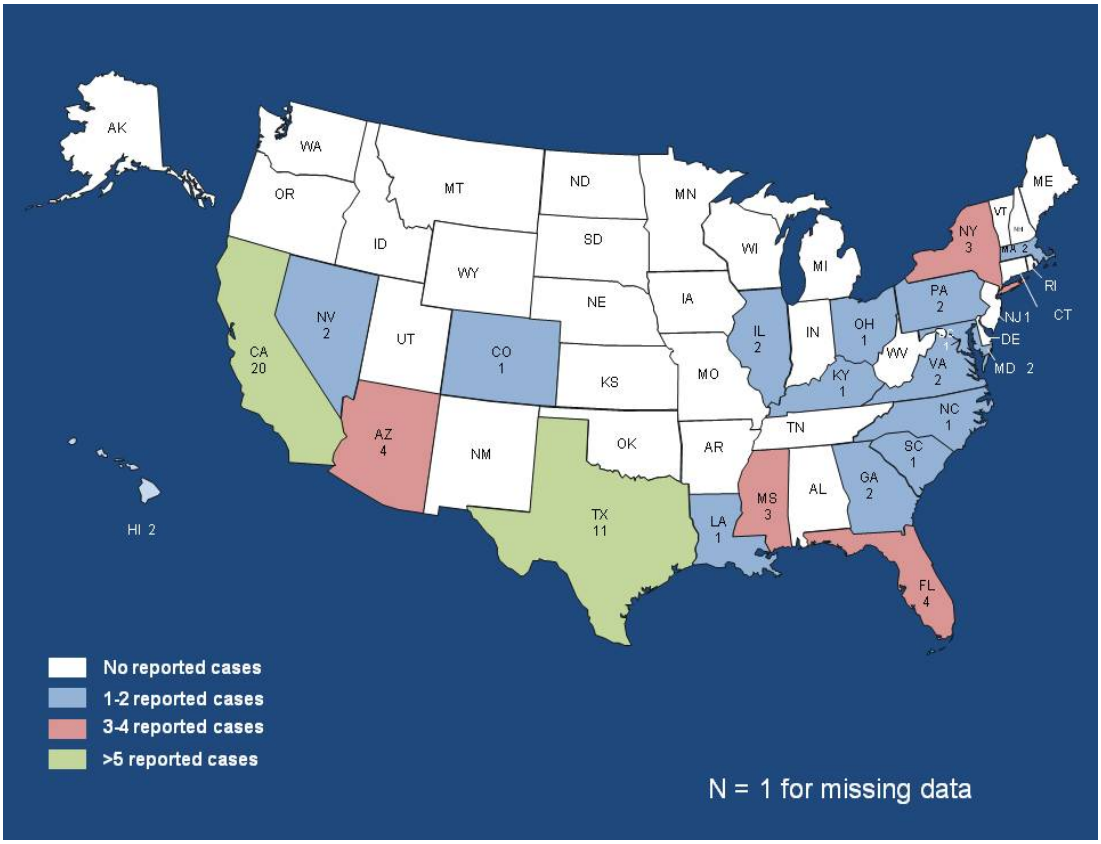


Table 1: Summary of Demographic Data for Non-Keratitis *Acanthamoeba* spp. and *Balamuthia mandrillaris* Cases

Variable		*Acanthamoeba* (N=114)		*Balamuthia* (N=70)	
		Data	Missing	Data	Missing
Race	White	63% (27/43)	62% (71/114)	58% (19/33)	53% (37/70)
	African American	35% (15/43)		36% (12/33)	
	American Indian	2% (1/43)		0% (0/33)	
	Asian/Pacific Islander	0% (0/43)		6% (2/33)	
Ethnicity	Hispanic	82% (9/11)	90% (103/114)	76% (22/29)	59% (41/70)
	Non-Hispanic	18% (2/11)		24% (7/29)	
Immigration	Immigrant	75% (6/8)	93% (106/114)	36% (4/11)	84% (59/70)
	Non-Immigrant	25% (6/8)		64% (7/11)	
Age	Range	8 m–79 y	4% (4/114)	4 m–89 y	3% (2/70)
	Mean	38.8 years		30.4 years	
Sex	Male	73% (82/112)	2% (2/114)	65% (45/69)	1% (1/70)

Presenting Symptoms: Research Question 2

Patients presented with a variety of non-descriptive symptoms for both *Acanthamoeba* and *Balamuthia* disease. Fever and headache were the two most common symptoms shared by those with either disease. See Tables 2 and 3 for the top ten symptoms patients presented with upon examination or hospitalization for illness caused by *Acanthamoeba* spp. and *Balamuthia mandrillaris* respectively.

Table 2: Top Ten Presenting Symptoms of Patients Later Diagnosed With Non-Keratitis *Acanthamoeba* spp. Infection (N=95/114)

Presenting Symptom	No. of Cases Reporting Symptoms
Other General Symptoms*	39
Fever	34
Headache	29
Sinus problems	18
Other Neurologic Deficit**	17
Lethargy/Fatigue	13
Confusion	9
Weight Loss	8
Weakness	7
Altered Mental Status	6
Cough	6
Nausea	6
Vomiting	6

*Other general symptoms include but were not limited to: chills (n=7/39), facial swelling/pain/tenderness (n=5/39), epitstaxis (n-=3/39), jaundice (n=2/39), night sweats (n=2/39), and incontinence (n=2/39).
** Other neurologic symptoms include but are not limited to: Romberg's sign (n=1/17), Babinski sign (n=1/17), Kernig's sign (n=1/17), Brudzinki's sign (n=1/17).

Table 3: Top Ten Presenting Symptoms of Patients Later Diagnosed With *Balamuthia mandrillaris* Infection (N=63/70)

Presenting Symptom	No. of Cases Reporting Symptoms
Fever	23
Headache	18
Lethargy/Fatigue	18
Vomiting	18
Other General Symptoms*	16
Other Neurologic Deficit**	15
Seizures	14

Weakness	13
Other Visual Symptoms	11
Altered Mental Status	10
Hemiparesis	9
Ataxia	8

*Other general symptoms include but were not limited to: incontinence (n=3/16), neck pain (n=2/16), and loss of appetite (n=2/16).
** Other neurologic symptoms include but are not limited to:Babinski sign (n=2/15, and unresponsiveness (n=2/15).

Because both acanthamoebiasis and balamuthiasis have multiple disease manifestations, a further exploration was done looking at presenting symptoms by disease manifestation. Symptoms were broken up into four categories: general presenting symptoms (17 variables), visual symptoms (4 variables), neurologic symptoms (29 variables), and skin lesions (4 variables). Cases that had no information for every variable in a category were taken out of the frequency counts and treated as unknowns. Remaining cases may have had more than one symptom in each of the four presenting symptom categories. In the case of the skin lesions category, knowns included those cases who indicated they had skin lesions or did not have skins lesions. After analysis it was apparent that not all of the possible variables for the symptom categories were selected as symptoms upon presentation. See Tables 4-7 for breakdowns of *Acanthamoeba* cases; see table footnotes for numbers of variables not chosen in each category.

Table 4: Presenting General Symptoms for Cases of Non-Keratitis *Acanthamoeba* spp. Infection by Disease Manifestation

Disease Manifestation	Presenting Symptom	No. of Known Cases Reporting Symptoms
Disseminated (n=30/36)*	Other General Symptoms	19
	Fever	12
	Sinus problems	11
	Headache	10
	Lethargy/Fatigue	5
	Cough	4

	Nausea	3
	Weight Loss	3
	Shortness of breath	2
	Vomiting	1
	Stiff Neck	1
GAE (n=25/48)*	Fever	16
	Headache	14
	Other General Symptoms	14
	Lethargy/Fatigue	7
	Vomiting	4
	Nausea	3
	Weight Loss	3
	Diarrhea	2
	Anorexia	1
	Cough	1
	Shortness of Breath	1
	Sinus Problems	1
Cutaneous (n=10/23)*	Fever	5
	Headache	3
	Other General Symptoms	4
	Diarrhea	2
	Weight Loss	2
	Myalgia	2
	Sinus Problems	2
	Shortness of Breath	1
	Lethargy/Fatigue	1
Rhinosinusitis (n=4/6)*	Sinus Problems	4
	Fever	1
	Headache	1
	Other General Symptoms	1
Osteomyelitis (n=1/1)	Other General Symptoms	1

*Those reporting multiple general symptoms on initial presentation included: 19 GAE cases, 24 disseminated cases, two rhinosinusitis cases, and six cutaneous cases. Although it was possible to select up to 17 variables for presenting general symptoms, three of those variables were not selected among those cases who reported presenting symptoms.

Table 5: Presenting Visual Symptoms for Cases of Non-Keratitis *Acanthamoeba* spp. Infection by Disease Manifestation

Disease Manifestation	Presenting Symptom	No. of Known Cases Reporting Symptoms
Disseminated (n=3/36)*	Blurred Vision	2
	Diplopia	1
	Other Visual Symptom	1
GAE (n=5/48)*	Blurred Vision	2
	Diplopia	2
	Other Visual Symptom	2
	Photophobia	1
Cutaneous (n=2)	Photophobia	2

*Those reporting multiple visual symptoms on initial presentation included: two GAE cases and one disseminated case.

Table 6: Presenting Neurologic Symptoms for Cases of Non-Keratitis *Acanthamoeba* spp. Infection by Disease Manifestation

Disease Manifestation	Presenting Symptom	No. of Known Cases Reporting Symptoms
Disseminated (n=12/36)*	Confusion	5
	Other Neurologic Deficit	4
	Weakness	3
	Altered Mental Status	3
	Behavioral Changes	1
	Dysphagia	1
	Hallucinations	1
	Seizures	1
	Hemiparesis	1
GAE (n=22/48)*	Other Neurologic Deficit	12
	Weakness	4
	Hemiparesis	3
	Seizures	3
	Confusion	3
	Altered Mental Status	2
	Aphasia	2
	Ataxia	2
	Numbness or Facial Numbness	1
	Hyperreflexia	1
	Nystagmus	1

Cutaneous (n=2/23)	Other Neurologic Deficit	1
	Altered Mental Status	1

*Those reporting multiple neurologic symptoms included: 10 GAE cases and four disseminated cases.
Although it was possible to select up to 29 variables for presenting neurologic symptoms, 15 of those variables were not selected among those cases who reported presenting symptoms.

Table 7: Presenting Skin Lesions for Cases of *Acanthamoeba* spp. Infection by Disease Manifestation

Disease Manifestation	**Type of Skin Lesion**	**No. of Known Cases Reporting Symptoms**
Disseminated (n=34/36)*	Other Type	16
	Ulcers	7
	Erythematous Nodules	6
	No Skin Lesions	7
GAE (n=7/48)	Other Type	3
	Ulcers	1
	No Skin Lesions	3
Cutaneous (n=23/23)*	Other Type	9
	Erythematous Nodules	7
	Ulcers	5
	Type Not Stated	4
Rhinosinusitis (n=2/6)	Ulcers	1
	No Skin Lesions	1

*Those reporting multiple types of skin lesions on initial presentation included: four disseminated cases and two cutaneous cases.
Although it was possible to select up to 4 variables for presenting skin lesions, one of those variables were not selected among those cases who reported presenting symptoms.

Although the manifestations of balamuthiasis appear to be limited to GAE and disseminated infection, which potentially have similar presenting symptoms, a breakdown by disease manifestation and the same four presenting symptom categories was done for cases of *Balamuthia mandrillaris*. This allows for an examination of any similarities or differences in manifestations as well as a comparison to *Acanthamoeba* GAE and disseminated disease. *Balamuthia* cases that had no information for any

variable in a category were taken out of the frequency counts and treated as unknowns. Remaining cases may have had more than one symptom in each of the 4 presenting symptom categories. See Tables 8-11 for presenting symptoms of *Balamuthia* GAE and disseminated balamuthiasis.

Table 8: Presenting General Symptoms for Cases of *B. mandrillaris* Infection by Disease Manifestation

Disease Manifestation	Presenting Symptom	No. of Known Cases Reporting Symptoms
Disseminated (n=5/8)*	Headache	3
	Nausea	2
	Vomiting	2
	Other General Symptoms	2
	Fever	1
	Diarrhea	1
	Weight Loss	1
	Stiff Neck	1
	Disorientation	1
	Lethargy/Fatigue	1
GAE (n=41/62)*	Fever	20
	Vomiting	16
	Headache	15
	Lethargy/Fatigue	15
	Other General Symptoms	14
	Nausea	5
	Anorexia	3
	Stiff neck	2
	Disorientation	2
	Diarrhea	1

*Those reporting multiple general symptoms on initial presentation included: 25 GAE cases and three disseminated cases.
Although it was possible to select up to 17 variables for presenting general symptoms, four of those variables were not selected among those cases who reported presenting symptoms.

Table 9: Presenting Visual Symptoms for Cases of *B. mandrillaris* Infection by Disease Manifestation

Disease Manifestation	Presenting Symptom	No. of Known Cases Reporting Symptoms
Disseminated (n=1/8)	Other Visual Symptom	1

GAE (n=13/62)*	Other Visual Symptom	10
	Diplopia	4
	Photophobia	1

*Those reporting multiple visual symptoms on initial presentation included: two GAE cases.
Although it was possible to select up to 4 variables for presenting visual symptoms, one of those variables were not selected among those cases who reported presenting symptoms.

Table 10: Presenting Neurologic Symptoms for Cases of *B. mandrillaris* Infection by Disease Manifestation

Disease Manifestation	**Presenting Symptom**	**No. of Known Cases Reporting Symptoms**
Disseminated (n=5/8)*	Other Neurologic Deficit	3
	Hemiparesis	2
	Seizures	2
	Altered Mental Status	1
	Aphasia	1
	Confusion	1
	Hallucinations	1
	Weakness	1
GAE (n=48/62)*	Other Neurologic Deficit	12
	Weakness	12
	Seizures	12
	Altered Mental Status	9
	Ataxia	8
	Hemiparesis	7
	Behavioral Changes	6
	Aphasia	5
	Confusion	4
	Cranial Nerve Deficit VI	3
	Other Cranial Nerve deficit	3
	Cranial Nerve Deficit VII	2
	Dysphagia	1
	Facial Numbness	1
	Hallucinations	1
	Combativeness	1
	Hyperreflexia	1
	Loss of Balance	1
	Nystagmus	1

Those reporting multiple neurologic symptoms on initial presentation included: 22 GAE cases and five disseminated cases.

Although it was possible to select up to 29 variables for presenting neurologic symptoms, 10 of those variables were not selected among those cases who reported presenting symptoms.

Table 11: Presenting Skin Lesions for Cases of *B. mandrillaris* Infection by Disease Manifestation

Disease Manifestation	**Type of Skin Lesion**	**No. of Known Cases Reporting Symptoms**
Disseminated (n=4/8)	Other type	3
	Ulcers	1
GAE (n=17/62)	Other type	3
	No Skin Lesions	14

Although it was possible to select up to 4 variables for presenting skin lesions, two of those variables were not selected among those cases who reported presenting symptoms.

In looking at a comparison of the top five presenting general symptoms for disseminated acanthamoebiasis and balamuthiasis (Table 12), three of the top five were the same. The differences were that disseminated acanthamoebiasis cases reported sinus problems and lethargy/fatigue and disseminated balamuthiasis did not. But *Balamuthia* cases experienced vomiting and nausea frequently enough to be in the top five general symptoms. For presenting neurologic symptoms, the top five symptoms were similar for both disseminated diseases but varied in that disseminated acanthamoebiasis cases reported behavioral changes and dysphagia. Disseminated acanthamoebiasis cases reported more visual symptoms more types of skin lesions than disseminated balamuthiasis cases.

Table 12: Comparison of Top Five Presenting Symptoms for Disseminated *Acanthamoeba* and *Balamuthia* Disease

General Symptoms			
Acanthamoeba (n=30/36)		*Balamuthia* (n=5/8)	
Presenting symptom	No. of Known Cases Reporting Symptoms	Presenting symptom	No. of Known Cases Reporting Symptoms
Other General	19	Headache	3

Symptoms			
Fever	12	Nausea	2
Sinus Problems	11	Vomiting	2
Headache	10	Other General Symptoms	2
Lethargy/Fatigue	5	Fever	2
Neurologic Symptoms			
Acanthamoeba (n=12/36)		*Balamuthia* (n=5/8)	
Confusion	5	Other Neurologic Symptom	3
Other Neurologic Symptom	4	Hemparesis	2
Weakness	3	Seizures	2
Altered Mental Status	3	Altered Mental Status, Aphasia, Confusion, Hallucinations, Weakness	Each symptom had a count of 1
Behavioral Changes, Dysphagia, Hallucinations, Seizures, Hemiparesis	Each symptom had a count of 1		
Visual Symptoms			
Acanthamoeba (n=3/36)		*Balamuthia* (n=1/8)	
Blurred Vision	2	Other Visual Symptom	1
Diplopia	1		
Other Visual Symptom	1		
Skin Lesions			
Acanthamoeba (n=34/36)		*Balamuthia* (n=4/8)	
Other Type	16	Other Type	3
Ulcers	7	Ulcers	1
Erythematous Nodules	6		
No Skin Lesions	7		

In comparing the top five presenting general symptoms for *Acanthamoeba* and *Balamuthia* GAE (Table 13), four of the five were the same. The only difference was that *Acanthamoeba* GAE cases reported nausea, whereas *Balamuthia* GAE cases reported vomiting. The presenting neurologic symptoms also differed somewhat, with 3 of the 5 being the same, but *Acanthamoeba* GAE cases reported hemiparesis and confusion, whereas *Balamuthia* GAE cases indicated having altered mental status and ataxia. *Acanthamoeba* GAE case-patients reported blurred vision where as *Balamuthia* GAE case-patients did not; *Balamuthia* GAE case-patients reported a high number of other visual symptoms. Both had reports of skin lesions but they were not diagnosed as cutaneous disease manifestations of *Acanthamoeba* or *Balamuthia*.

Table 13: Comparison of Top Five Presenting Symptoms for *Acanthamoeba* and *Balamuthia* GAE

General Symptoms			
Acanthamoeba (n=25/48)		*Balamuthia* (n=41/62)	
Presenting symptom	No. of Known Cases Reporting Symptoms	Presenting symptom	No. of Known Cases Reporting Symptoms
Fever	16	Fever	20
Headache	14	Vomiting	16
Other General Symptom	14	Headache	15
Lethargy/Fatigue	7	Lethargy/Fatigue	15
Nausea	4	Other General Symptom	14
Neurologic Symptoms			
Acanthamoeba (n=22/48)		*Balamuthia* (n=48/62)	
Other Neurologic Symptoms	12	Other Neurologic Symptoms	12
Weakness	4	Weakness	12
Hemiparesis	3	Seizures	12
Seizures	3	Altered Mental Status	9
Confusion	3	Ataxia	8
Visual Symptoms			

Acanthamoeba (n=5/48)		*Balamuthia* (n=13/62)	
Blurred Vision	2	Other Visual Symptom	10
Diplopia	2	Diplopia	4
Other Visual Symptom	2	Photophobia	1
Photophobia	1		
Skin Lesions			
Acanthamoeba (n=5/48)		*Balamuthia* (n=13/62)	
Other Type	3	Other Type	3
Ulcers	1	No Skin Lesions	14
No Skin Lesions	3		

Treatment: Research Question 3

The survivors of *Acanthamoeba* infection were cases whose disease manifestations were cutaneous, rhinosinusitis or disseminated infection, typically involving skin and sinuses. There has not been a reported case of *Acanthamoeba* GAE, or disseminated acanthamoebiasis with GAE involvement, that has survived. Among the survivors of *Acanthamoeba* disease of those with known outcomes (n=9/86), there was no common drug used in all cases; however, five cases took itraconzole and four cases took pentamidine (Table 14). Drug commonalities by *Acanthamoeba* disease manifestation are also summarized in Table 14. Non-survivors also took many of the same drugs. Cases that had no information for any drug listed in the database, or that had unknown outcomes, were taken out of the frequency counts and treated as unknowns. Among *Balamuthia* survivors (n=6), there were also no common drugs used in all cases, although fluconazole and pentamidine was used in all but one instance (Table 15). Drug commonalities by *Balamuthia* disease manifestation are also summarized in Table 15. Again, non-survivors also took many of the same drugs.

Table 14: Drugs in Common for Non-Keratitis *Acanthamoeba* Survivors Compared to Non-Survivors

	Drug	No. of Survivors With Known Treatment	No. of non-Survivors with Known Treatment
All manifestations	Itraconazole*	5/9	6/47
	Pentamidine	4/9	11/47
	Ketoconazole	3/9	6/47
	Chlorhexidine topical	3/9	3/47
	Amphotericin B	2/9	14/47
	Flucytosine	2/9	6/47
	Voriconazole	2/9	5/47
Disseminated	Itraconazole	2/3	4/21
	Ketoconazole	2/3	3/21
	Pentamidine	2/3	5/21
	Chlorhexidine topical	2/3	1/21
GAE		No Survivors	
Cutaneous	Itraconazole	2/3	2/7
	Pentamidine	2/3	4/7
Rhinosinusitis		No Drugs in Common for Survivors (n=2)	
Osteomyelitis		1 case	

Table 15: Drugs in Common for *Balamuthia* Survivors Compared to Non-Survivors

	Drug	No. of Survivors With Known Treatment	No. of non-Survivors with Known Treatment
All manifestations	Fluconazole*	5/6	7/40
	Pentamidine*	5/6	6/40
	Clarithromycin*	4/6	1/40
	Sulfadiazine*	4/6	6/40
	Flucytosine*	4/6	4/40
	Azithromycin*	3/6	3/40
	Ketaconazole	2/6	0/401
Disseminated		1 case	
GAE	Azithromycin	2/5	3/35

	Clarithromycin	3/5	1/35
	Fluconazole	4/5	5/35
	Flucytosine	3/5	4/35
	Pentamidine	4/5	5/35
	Sulfadiazine	3/5	4/35

See Appendix C for depictions of treatment regimens over time for survivors.

Duration of Illness: Research Question 4

For case-patients who were diagnosed as having *Acanthamoeba* sp. infection and who were known to have died (n=77/114), 49% (n=38/77) had missing information on the number of days between illness onset and death. Of the decedents with known durations of illness (n=39/77), the mean duration of for illness (onset until death) was 101. The range was from 3 to 765 days. For case-patients with *Balamuthia* infections who were known to have died (n=61/70), 39% (n=24/61) had missing information on the number of days between illness onset and death. Of the decedants with known durations of illness (n=37/61) the mean duration of illness (onset until death) was 74 days, with a range of 8 days to 568 days. Based on the data that was seen during the collection and abstraction work, it appeared that the timing of diagnosis was often quite late in the course of illness or post-mortem.

Further Data Analysis

Exposures

There was a substantial lack of exposure data for both *Acanthamoeba* and *Balamuthia* cases. Only 6% (n=7/114) of *Acanthamoeba* cases had information about water exposured, six had exposures and one did not. Only 4% (n=4/114) had information about soil exposures; all four cases reportedly had exposure. One case reported an occupational exposure where the individual worked with animals. Only 20% (n=23/114)

of *Acanthamoeba* cases had information about travel and only two out of these included information about their travel. One case-patient reportedly visited Mexico and one was noted to have "traveled extensively," with no further details provided.

Only 17% (n=12/70) *Balamuthia* cases had information about water exposures; nine had exposures and three did not. Only 20% (n=14/70) had information about soil exposure; 12 cases had known exposures whereas two did not. Fifteen (21%) had occupational exposure information with four reporting actual occupations (three were farmers/ranchers or living on a farm, one was a firefighter). Half (n=35/70) of the cases had information about travel history and, of those, 12 gave travel location information and 23 did not. The country cited most frequently in the travel history was Mexico (7 cases).

Medical History

HIV status was reported for 58% (n=66/114) of *Acanthamoeba* cases; a minimum of 52% (n=59/114) of patients who became infected with *Acanthamoeba* spp. had HIV. Patients' AIDS status was known for 53% of cases (n=60/114); a minimum of 47% (n=54/114) of patients with *Acanthamoeba* disease had AIDS, of which 81% (n=44/54) had CD4 counts or another illness that indicated AIDS and 19% (n=10/54) did not.

Past medical history data, when available, also showed a variety of respiratory illnesses, other immunocompromising conditions, and prescription and illegal drug use, all of which might have predisposed patients to infection with *Acanthamoeba* spp. (Table 16). Twelve cases had no information on past medical history.

Table 16: Past Medical Conditions That May Be Predisposing Factors for Non-Keratitis *Acanthamoeba* spp. Infection (N=102/114)

	No. of Cases Reporting Condition
Respiratory Conditions	
Pneumonitis	30
Sinusitis	14
Other	5
Deviated Septum	2
TB	2
Nasal Surgery	1
Pharyngitis	1
Immunocompromising Conditions	
HIV	59
Organ transplant	22
Cancer	18
Diabetes	8
Renal failure	7
Alcohol misuse	5
Lymphoproliferative disease	5
Other autoimmune disease	4
Systemic lupus erthematosus	3
Other hematologic disease	3
Cirrohosis	1
Treatment/drugs	
Steriod use	17
Immunosuppressants	16
Eye Infections	7
Illegal drug use	6
Excessive antibiotic use	1
Skin infections	1

Because HIV/AIDS seemed to be so frequent among the *Acanthamoeba* cases, a further breakdown of other immunocompromising illnesses was done by HIV status. See Table 17.

Table 17: Positive Non-Keratitis *Acanthamoeba* Patients Reporting Another Immunocompromising Condition by HIV Status

Immunocompromising Conditions	**No. of HIV-Positive Cases Reporting Another Condition (n=7/59)***	**No. of HIV-Negative Cases Reporting Another Condition (n=7/7)****	**No. of HIV Unknown Cases Reporting Another Condition (n=27/48)*****
Cancer	3	2	13
Alcohol Misuse	2	2	1
Other Hematologic Disease	2	0	1
Renal failure	1	1	5
Other Autoimmune Disease	1	1	2
Organ Transplant	0	5	17
Diabetes	0	2	6
Systemic Lupus Erythematosus	0	1	2
Lymphoproliferative Disease	0	0	4
Cirrhosis	0	0	1

*Two HIV-positive *Acanthamoeba* cases had multiple immunocompromising conditions.
**Five HIV-negative cases had multiple immunocompromising conditions.
*** Eighteen cases had multiple immunocompromising conditions.
Other hematologic disease included: end-stage renal disease, hemophilia, and myelodysplastic syndrome.
Other autoimmune disease included: progressive transverse myelitis, graft vs. host disease, hemolytic anemia, and discoid lupis; hypogammaglobullnemia.

Unlike *Acanthamoeba* spp. infection, *Balamuthia* cases did not typically have HIV. Fifty-six percent of cases had an unknown HIV status (n=39/70). Of the 31 cases with a known status, only 26% (n=8/31) were HIV positive and 74% (n=23/31) were negative. Of the eight cases that had HIV, four had progressed to AIDS. See Table 18 for previous medical history that may predispose patients to infection with *Balamuthia mandrillaris*. Twenty-four cases had no information on past medical conditions.

Table 18: Past Medical Conditions That May Be Predisposing Factors for *Balamuthia mandrillas* Infection (N=46/70)

	No. of Cases Reporting Condition
Respiratory Conditions	
Otitis	6
Pharyngitis	3
Pneumonitis	3
TB	3
Other	2
Deviated Septum	1
Nasal surgery	1
Sinusitis	1
Immunocompromising Conditions	
Alcohol misuse	9
HIV	8
Diabetes	4
Organ transplant	3
Renal failure	3
Other hematologic disease	2
Cirrohosis	1
Treatment/drugs	
Illegal drug use	14
Skin infections	3
Steriod use	3

Initial and Final Diagnoses and Outcomes

Seventy-eight percent of *Acanthamoeba* cases had missing data for admitting diagnosis (n=89/114). Rarely, 8% of the time, were cases diagnosed with meningitis (n=1/25) or meningoencephalitis (1/25): such cases were typically diagnosed as other illness (92%, n=23/25) such as toxoplasmosis (n=3/23), toxoplasmosis vs. or with lymphoma (n=3/23), or vasculitis (n=3/23). The remaining initial diagnoses (n=14/23) were varied. Final diagnosis of *Acanthamoeba* cases are shown below in Table 19.

Table 19: Final Diagnosis of Cases of Non-Keratitis *Acanthamoeba* spp. Infection

	No. of Cases	Percent
Acanthamoeba GAE	48	42%
Disseminated acanthamoebiasis	36	32%
Acanthamoeba rhinosinusitis	6	5%

Cutaneous acanthamoebiasis	23	20%
Other: *Acanthamoeba* osteomyelitis	1	1%
Total	114	100%

Forty percent of *Balamuthia* cases had missing data for admitting diagnosis (n=28/70). As with *Acanthamoeba* infection, *Balamuthia* cases were rarely (26%, n=12/42) diagnosed with encephalitis (n=3/12), meningitis (n=6/12), or meningoencephalitis (n=3/12) on initial presentation, and more commonly diagnosed with other illnesses (74%, n=30/42) such as cysticercosis/neurocysticercosis (n=3/30), toxoplasmosis (n=3/30), stroke (n=2/30), neoplasia (n=3/30), or acute disseminated encephalomyelitis (ADEM) (n=2/30). The remaining initial diagnoses (n=25/30) were varied. Final diagnosis of *Balamuthia* cases are shown below in Table 20.

Table 20: Final Diagnosis of Cases of *Balamuthia mandrillaris* Infection

	No. of Cases	**Percent**
Balamuthia GAE	62	89%
Disseminated balamuthiasis	8	11%
Total	70	100%

Outcomes for both *Acanthamoeba* and *Balamuthia* infections were poor. Twenty-eight cases of *Acanthamoeba* had unknown outcomes (25%, n=114). Of the cases with known outcomes, 10% of case-patients survived (n=9/86) and 90% died (n=77/86). Prognosis for those with *Balamuthia* infection was similar. Three cases had unknown outcomes (4%, n=70). Of the cases with known outcomes, 9% survived (n=6/67) survived and 91% died (n=61/67).

CHAPTER V: DISCUSSION AND CONCLUSION

In examining the demographics of both *Acanthamoeba* and *Balamuthia* cases, the fact that the disease manifestations are far more common in men than women was one of the only conclusions that could be drawn from the data. The completeness of answers for this question was almost 100% in both *Acanthamoeba* and *Balamuthia* datasets. Similarly, the data on age was more than 96% complete for both groups. The vast majority (87%) of *Acanthamoeba* cases occurred in adults aged 18–64 years with a mean age of 38.8 years. However, the pattern for *Balamuthia* cases tended towards younger persons; while most (47%) were still adults 18–64 years of age, a large proportion of *Balamuthia* cases (43%) occurred in children younger than 18 years of age.

The scarcity of ethnicity data makes it difficult to interpret an epidemiologic pattern of this variable. More than 90% of the ethnicity data for *Acanthamoeba* cases was missing. While 82% (n=9/11) of patients with known ethnicity were Hispanic, a denominator of 11 is insufficient to draw conclusions. For *Balamuthia* cases, 59% of the ethnicity data was unknown. As with *Acanthamoeba* case-patients, while Hispanics did make up the majority, 76% (n=22/29), when the ethnicity of the *Balamuthia* case-patients was known, a denominator of 29 may not be enough to draw conclusions from. Schuster, Glaser, Honarmand, Maguire, and Visvesvara (2004) proposed that Hispanics may be at increased risk for *Balamuthia mandrillaris* infection based on the results of seven positive tests, all in Hispanic patients, conducted by the California Encephalitis Project (CEP) and because 8 of 11 cases in the State occurred in Hispanic Americans.[20] The

authors also searched CDC records and state that "approximately 50% of the 50 North American patients" were Hispanic; this number is potentially biased as it is based on the assumption that certain surnames were ethnically Hispanic. Also, because the California State Health Department has the CEP, data from that state may over-represent Hispanics because there are a high number of persons of that ethnicity in the state (37% based on U.S. Census estimates in 2009).[29]

However, some suggestion of an ethnicity bias is present in the *Balamuthia* data. This thesis also examined case records at CDC and in the published literature. In looking at the national proportion of Hispanic ethnicity in 1999, the median year of the *Balamuthia* dataset, it was11.5%.[30] Even though the ethnicity was unknown for 41 *Balamuthia* case-patients, 22 (31%) of the total *Balamuthia* cases (n=70) were of known Hispanic ethnicity, which is already greater than the population proportion. And while the suggestion that balamuthiasis might be more common in Hispanics versus non-Hispanics may be true, the cause of this possible association is unknown and might reflect a number of factors, possibly including different exposure patterns.

In looking at the geographic distribution of cases, there is no apparent pattern by state of treatment, with cases being treated in all regions of the continental United States. For both *Acathamoeba* and *Balamuthia*, those states reporting more cases tended to be the states with larger State health departments. Although exposure patterns might be different in some states, it's worth considering that the large number of cases reported in California might be due to its ongoing CEP. Similarly the large number of cases from Texas might be because of its proximity to and awareness of the CEP's work. Because the CEP conducts active surveillance on cases of encephalitis, it may be that the numbers

of free-living ameba cases from California and Texas indicate that cases are more frequent than supposed and are being diagnosed because the cause of encephalitis is being closely looked at.

Presenting symptom information is not encouraging in terms of gaining a better understanding of how to more rapidly suspect and thereby diagnosis and treat free-living amebae infection. Much of the presenting symptom information was missing from cases diagnosed with *Acanthamoeba* GAE, ranging from 48% missing on general symptoms to 90% missing visual symptoms. Gaps in visual symptom and neurologic symptom data were also apparent for disseminated acanthamoebiasis cases and in each symptom category for cutaneous cases, with the exception of presenting skin lesions. Even among the cutaneous cases, the type of lesion seen on presentation was highly variable and not pathognomonic. While both *Acanthamoeba* GAE and disseminated acanthamoebiasis had fever and headache as the predominant general symptoms and other neurologic deficits and weakness were typical neurologic symptoms, these symptoms and signs are rather vague symptoms and can indicate many types of illness. It may be that there are specific neurologic deficits that could stand out if the data were more complete.

In contrast, cases diagnosed with *Balamuthia* GAE or disseminated balamuthiasis had better completion of data for both presenting general symptoms (34% missing) and neurologic symptoms (23% missing). Similar to *Acanthamoeba*, headache was common among GAE and disseminated cases, but vomiting also was high on the list of common symptoms, with fever more evident in GAE cases than in disseminated cases. Interestingly, unlike *Acanthamoeba*, infection with *Balamuthia* was not noted to have any symptoms related to sinus problems, nor were there documented cases of *Balamuthia*

rhinosinuitis. Also there were no standalone cases of cutaneous balamuthiasis, and skin manifestations only occurred in cases of disseminated infections. All disseminated cases had granulomatous amebic encephalitis and a second disease manifestation (most often skin), whereas disseminated infections of acanthamoebiasis were more varied in their manifestations with only 11 of the 30 cases having proven GAE.

The generality of signs and symptoms for both *Acanthamoeba* and *Balamuthia* are reflected in the initial diagnoses. Although the data were limited, the classical diagnoses for headaches and fevers (i.e., meningitis, encephalitis, and meningoencephalitis) were not commonly made. Rather, other diagnoses were more common, including: parasitic diseases (e.g., toxoplasmosis, cysticercosis, neurocysticercosis), neoplasias, vasculitis, or stroke. The variables other neurologic symptoms, other general symptoms, and other visual symptoms should be more closely examined to determine if there any signs or symptoms for these variables that suggest a unique symptom for free-living ameba infection.

Examination of the treatment regimens in survivors of acanthamoebiasis shows that while there were several common drugs administered to the survivors, the same drugs were used in non-survivors. A closer examination of the combination of drugs used needs to be done to determine if there is a combined therapy that is more effective. Balamuthiasis differs in that the two manifestations of the disease reported have always involved the central nervous system. As with acanthamoebiasis, drugs in common usage for the survivors were often used in non-survivors. Again, closer examination of the combination of drugs used needs to be done. Duration and dosage are also important factors, but unfortunately much of the dosage information or dosage timing is missing

from this retrospective review. So going forward in data collection for an informal surveillance system of free-living amebas, it is essential that accurate treatment information is collected for closer examination of whether or not certain drugs are more effective than others.

The mean number of days from illness onset to death for cases of *Balamuthia mandrillaris* was 74 and for *Acanthamoeba* spp. was 100 days. This seems to indicate that there is sufficient time to make a correct diagnosis, but further evaluation of the data is necessary to examine the timing of hospitalization, timing of a correct diagnosis, and timing of treatment specific to free-living ameba infection. If it is determined that sufficient time exists for a correct diagnosis, then the question becomes how to increase awareness of these infections so they are included in a differential diagnosis when patients present with symptoms that tend to be very common to various forms of meningitis, and where other potential diagnoses such as toxoplasmosis, neurocysticercosis, and tumors are often considered far more quickly.

Outside of the main research questions, much of the other data explored was inconclusive. The epidemic curve for *Acanthamoeba* spp. infection shows the highest number of cases between 1994 and 1997 with an apparent drop off in reported cases after 2003. Because the infection is rare, this is difficult to interpret. The increase of cases in the 1990s is likely due to greater awareness of the infection and increased requests to the Centers for Disease Control and Prevention for diagnostic assistance. Are the cases really decreasing, or are other laboratories beginning to perform diagnostic testing and therefore CDC is not receiving specimens or reports? It is important to continue to monitor cases to see if there truly is this drop in cases or if the number of infections

remains relatively constant over time. Because *Balamuthia* was not recognized as the cause of human infections until around 1990, the epidemic curve is not particularly surprising, with some fluctuations throughout the 1990s and then a seeming increase in cases in more recent years, again likely due to greater awareness of free-living ameba infections.

The exposure data were too scarce to draw conclusions from in terms of modes of transmission (i.e., water, soil). While persons with *Naegleria fowleri* often have a history of swimming and recreational water activities in freshwater, persons with *Acanthamoeba*, where exposure history was known, had both water and soil exposures, as did those with *Balamuthia*. The types of water bodies, water conditions, and recreational water activities are data that are not known in these cases.

The data did strongly support the supposition that *Acanthamoeba* infection disproportionately affect immunocompromised persons. Outside of those with HIV, it appears that steroids and immunosuppressant drugs may be important conditions. Although not necessarily associated with immunosuppression, a history of pneumonitis also seemed to be common. This might reflect a compromised portal of entry for the pathogens or perhaps pneumonitis is a common co-morbidity in those *Acanthmaoeba* patients with AIDS. The past medical history for *Balamuthia mandrillaris* cases is less revealing, although drug and alcohol abuse appeared to be a relatively common potentially immunosuppressive condition as well as a variety of respiratory conditions.

The data exploration for this thesis was limited and the data collected for CDC's informal surveillance system for free-living amebas has more information to be accessed and analyzed. It will be important, as noted above, to look closer at the timing of onset,

hospitalization, diagnosis, and death or survival in hope that there may be some information that might be useful in improving clinical outcomes. Because so little data on U.S. cases is available, fewer than 200 cases with much missing data, it is crucial to gather as much information as possible about exposure, presentation, and treatment of future cases. This exploration has been the first comprehensive look at all known cases of *Acanthamoeba* and *Balamuthia* infection in 20 years and it contributes to a broader understanding of these illnesses.

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Appendix A: Detailed Tables of All Published Non-Keratitis *Acanthamoeba* and *Balamuthia* Cases

Table 1: Disseminated Acanthamoebiasis

Case (Ref)	Yr	State	Age (yr)	Sex	Race /Ethnicity	Health Status	Type of Illness	Clinical Course in Days*	Died
1[21,27,31-33]	1972	TX	7	M	Black/ Unknown	Pharyngitis, water & soil exposure	GAE, keratitis	~44 (s); 7 (h)	Y
2[6,17,21,27,34]	1977	CA	24	F	Unknown/ Hispanic	Unknown	GAE, skin, sinus, lymph node, adrenals, thyroid	~229 (s); ~49 (h)	Y
3[6,35]	1978	UT	57	F	Unknown/ Unknown	Systemic lupus erthematosus	GAE, lungs, skin	~8 (s); ~3 (h)	Y
4[6,36]	1980	PA	38	M	White/ Unknown	Kidney transplant	GAE, skin, pulmonary hyaline membrane	~43 (h); [Possibly 720 days (s)]	Y
5[22,24,37-40]	1983	FL	29	M	Unknown/ Unknown (Haitian)	AIDS	sinus, skin	~138 (s); 108 (h)	Y
6[6,22,23,39,41]	1985	CA	34	M	Uknown/ Unknown (Mexican American)	AIDS	GAE, skin	~9 (s); ~2 (h); [Possibly 374 days (s)]	Y
7[6,40,42]	1987	CA	35	M	Black/ Unknown	AIDS, PCP, IVDA	skin, keratitis, lungs	~92 after diagnosis; 8 (h)	Y
8[6,24,43]	1989	OH	34	M	Unknown/ Unknown	AIDS, recurrent otitis	skin, sinus	~210 (s)	Y
9[6,22,23,44]	1989	IL	38	M	White/ Unknown	AIDS, PCP	GAE, skin	~24 (h)	Y
10[39,45]	1990	MA	39	M	Unknown/ Unknown	AIDS	GAE, lungs	~8 (s); 4 (h)	Y
11[22,24,39,40,46]	1990	PA	7	M	Unknown/ Hispanic	HIV, demyelinating polyneuropathy	skin, sinus	~193 (s)	Y

12[47]	1991	TX	39	F	Unknown/ Unknown	CML, bone marrow transplant, pneumonia	GAE, lungs, adrenalitis	~25 (s)	Y
13[22,24, 39,40,48]	1991	OR	33	M	Unknown/ Unknown	AIDS, PCP, sinusitis	skin, sinus	~123 (s) until lesions resolved, treatment con't for 8 mo. after	Y
14[22,40, 42]	1992	CA	47	M	Unknown/ Hispanic	AIDS, PCP, HIV wasting sickness	skin, sinus	~120 (h)	Y
15[22,40, 42]	1992	CA	36	M	American Indian/ Unknown	AIDS, PCP	skin, sinus	~120 (h)	Y
16[40,49]	1993	TX	.8	M	Black/ Unknown	HIV	skin, lungs, lymph node, heart, kidney, spleen	~765 (s)	Y
17[22,50]	1994	Unkn own	5	M	Black/ Unknown	HIV, PCP	skin, osteo-myelitis	~810 (s), with occasional skin lesions recurring	N
18[22,40, 42]	1994	CA	45	M	White/ Unknown	AIDS, PCP, chronic sinusitis	skin, sinus, bone	~120 (h)	Y
19[22,40, 42]	1994	CA	34	M	White/ Unknown	AIDS, PCP, chronic sinusitis	GAE, skin, sinus	~111 (s)	Y
20[22,34, 47]	1994 ?	TX	32	F	Unknown/ Unknown	AML, bone marrow transplant, sinusitis	GAE, skin	~18 (s)	Y
21[51]	1995	NY	33	M	Black/ Unknown	AIDS, HIV-related cerebral atrophy	skin, sinus	~42 (s); tx for another 150 days until death	Y
22[24,40]	1995	MA	37	M	Unknown/ Unknown	AIDS	skin, sinus	Min ~90 (s)	Unk now n
23[34,52]	1997	SC	39	F	Black/ Unknown	Lung transplant	skin, liver, BAL	~124 (s); 118 (h)	N
24[53]	2000	Unkn own	37	M	Unknown/ Unknown	AIDS, non-Hodgkins lymphoma	skin, sinus	~131, tx continued after with no evidence of recurrent infection	N

25[40,54]	2000	Unknown	42	M	Unknown/ Unknown	AIDS, peripheral neuropathy	sinus, lungs	~19 (s); 16 (h)	Y
26[55]	2001	GA	45	M	Unknown/ Unknown	Acute myelogenous leukemia, stem cell transplant	GAE, lung	~98 (s); 3 (h)	Y
27[34]	2001	MD	61	F	Unknown/ Unknown	Kidney transplant, renal failure	skin, bone	~26+ (h)	Y
28[22,40,56]	2001	FL	35	F	Unknown/ Unknown (Nicaraguan)	AIDS, recurrent sinus surgery	skin, sinus	Unknown	Y
29[57,58]	2003	TX	60	M	White/Unknown	Bilateral lung transplant; water exposure	GAE, skin, lung	~38 (s); 17 (h)	Y

*Clinical course may mean from symptom onset until death or resolution of illness (s) or from presentation/hospitalization until death or resolution of illness (h).
AIDS = Acquired Immune Deficiency Syndrome; HIV = Human Immunodeficiency Virus; PCP = *Pneumocystis carinii* pneumonia, IVDA = intravenous drug abuse; CML = chronic myelogenous leukemia; AML = acute myeloid leukemia

Table 2: Disseminated Balamuthiasis

Case (Ref)	Yr	State	Age (yr)	Sex	Race/ Ethnicity	Health Status	Type of Illness	Clinical Course in Days	Died
1[6,17,59]	1975	LA	56	M	Black/ Unknown	Alcohol misuse, G6PD	GAE, pancreas	~27 (s); 13 (h)	Y
2[6,16,23,60,61]	1985	NY	36	M	Unknown/ Unknown	HIV, IVDA	GAE, kidney, adrenals	~25 (s); ~18 (h)	Y
3[16,23]	1989	GA	52	M	Unknown/ Unknown	Amputation, soil exposure	GAE, cutaneous	~101 (s); ~77 (h)	Y
4[62]	1996	CA	64	M	White/ Unknown	Ankylosing spondylitis, gardening	GAE, cutaneous	Unknown	N
5[63]	1999	CA	2	F	Unknown/ Hispanic	Otitis media	GAE, lungs	~24 (s); 9 (h)	Y
6[64]	1999	TX	38	M	Unknown/ Hispanic	IVDA	GAE, cutaneous	~568 (s); 19 (h)	Y
7[65]	1999	TX	89	M	Unknown/ Unknown	Coronary artery disease, mild congestive heart failure	CSF, cutaneous	~390 (s); 13 (h)	Y

*Clinical course may mean from symptom onset until death or resolution of illness (s) or from presentation/hospitalization until death or resolution of illness (h).

HIV = Human Immunodeficiency Virus ; G6PD = glucose-6-phospate dehydrogenase deficiency; IVDA = intravenous drug abuse

Table 3: *Acanthamoeba* GAE

Case (Ref)	Yr	State	Age (yr)	Sex	Race/ Ethnicity	Health Status	Clinical Course in Days	Died
1[6,17,21,26,27]	1955	AZ	6	F	Unknown/ Unknown	Unknown	~252 (s); 16 (h)	Y
2[66]	1964	TX	59	M	Unknown/ Unknown	Drug abuse, cirrhosis	21 (h)	Y
3[6,17,27,67]	1971	PA	58	M	Unknown/ Unknown	Alcohol abuse, pneumonia, water exposure	~21 (h)	Y
4[6,27,68]	1971	NY	57	M	Unknown/ Unknown	History of stroke, intestinal amebiasis, water exposure	Unknown	Y
5[6,14,15,17,27]	1972	NY?	27	M	White/ Unknown	Hodgkins, long-term immune-suppression, mild respiratory infections	31 (s); 5 (h)	Y
6[6,17,21]	1976	LA	32	F	White/ Unknown	Pneumonitis	~43 (s)	Y
7[6]	1978	NY	11	F	Unknown/ Unknown	Unknown, soil exposure	Unknown	Y
8[6]	1978	TX	2	F	Unknown/ Unknown	Unknown	Unknown	Unknown
9[6,22,23]	1981	GA	31	F	Unknown/ Unknown	Mixed connective tissue disorder	~40 (s); 9 (h)	Y
10[6,23,39,69]	1985	CA	34	M	Unknown/ Unknown	HIV, PCP	~35 (s); ~28 (h)	Y
11[6,23,39]	1987	GA	34	M	Unknown/ Uknown	AIDS, PCP	8 (s); 4 (h)	s
12[6]	1988	MN	60	M	Unknown/ Unknown	Unknown	Unknown	Unknown
13[6]	1988	SC	38	M	Unknown/ Unknown	Unknown	Unknown	Unknown
14[23,39]	1990	GA	34	M	Unknown/ Unknown	AIDS, PCP	~15 (s); 8 (h)	Y
15[70]	1997 ?	NM	35	M	Unknown/ Unknown	IVDA, alcohol abuse	~11 (s)	Y
16[71]	1997	CT	47	F	Unknown/ Unknown	Moncytoid B cell lymphoma, stem cell transplant	~143 (s); 11 (h)	Y
17[72]	2003	PA	40	M	Unknown/ Unknown	Multiple visceral transplant, immune-suppressants	~14 (s); 12 (h)	Y
18[73,74]	2003	TN	70	F	White/ Unknown	Discoid lupus erthematosus	~99 (s); 63 (h)	Y

19[75]	2004	Unknown	51	M	White/ Unknown	Kidney transplant, diabetes, potential water exposure	~54 (s)	Y
20[76]	2005	VA	41	M	Black/ Unknown	HIV	~9 (s)	Y
21[77]	2006 ?	MA	24	F	Unknown/ Unknown	Systemic lupus erythematosus, drug and alcohol dependency	~120 (s); 60 (h)	Y

*Clinical course may mean from symptom onset until death or resolution of illness (s) or from presentation/hospitalization until death or resolution of illness (h).
AIDS = Acquired Immune Deficiency Syndrome; HIV = Human Immunodeficiency Virus; PCP = *Pneumocystis carinii* pneumonia, IVDA = intravenous drug abuse

Table 4: *Balamuthia* GAE

Case (Ref)	Yr	State	Age (yr)	Sex	Race/ Ethnicity	Health Status	Clinical Course in Days	Died
1[6,17,27,28]	1974	VA	47	F	Black/ Unknown	Diabetes, well water	~21 (s); 7 (h)	Y
2[6,16,78,79]	1978	SC	.4	M	White/ Unknown	Seizure activity, pneumonia	~35 (s); 5 (h)	Y
3[6,16,79,80]	1979	PA	2.5	M	Unknown/ Unknown	Unknown	240 (s)	Y
4[6,16,79]	1982	CA	.8	F	Unknown/ Unknown	Unknown	15 (s)	Y
5[6,16]	1983	FL	61	M	Unknown/ Unknown	Alcohol misuse, TB	112 (s)	Y
6[6]	1984	CA	72	M	Unknown/ Unknown	Unknown	Unknown	Y
7[6,16,79,81]	1986	TX	11	F	Unknown/ Hispanic	Healthy, soil exposure	150 (s)	Y
8[16]	1990	NV	60	M	Unknown/ Unknown	Alcohol misuse	Unknown	Y
9[82]	1990	NC	1.5	M	Unknown/ Unknown	Unknown, water exposure	~23 (h)	Y
10[83]	1991	TX	.11	F	Unknown/ Hispanic	Healthy	~28 (s)	Y
11[84]	1991	NV	.6	F	Unknown/ Unknown	Otitis, pharyngitis	~25 (h)	Y
12[79]	1992	AZ	13	F	Unknown/ Unknown	Healthy	~16 (s)	Y
13[85]	1993	TX	5	M	White/ Unknown	Healthy	~57 (s)	Y
14[79]	1993	AZ	2.3	M	Unknown/ Unknown	Otitis	~23 (s)	Y
15[85]	1993	TX	15	M	Unknown/ Hispanic	Healthy	~72 (s)	Y
16[86]	1995	MD	34	F	Unknown/ Unknown	HIV	~24 (h)	Y
17[87,88]	1996	CA	32	M	Unknown/	IVDA,	~42 (s)	Y

					Hispanic	alcohol misuse		
18[89]	1996	CO	3	M	Unknown/ Unknown	Pharyngitis	~17 (s)	Y
19[90]	1999	MA	52	F	Unknown/ Unknown	Chronic sinus infections, chronic idiopathic neutropenia	~105 (s)	Y
20[91]	1999	VT	5	F	Unknown/ Unknown	Healthy, water exposure	75 (h)	Y
21[62,88]	2000	CA	5	F	Unknown/ Hispanic	Healthy	~485 (h); multiple hospitalizations	N
22[7,8,63,92,93]	2001	CA	3	F	White/ Hispanic	Otitis	~24 (s)	Y
23[63]	2001	TX	2	M	Unknown/ Hispanic	Unknown	Unknown	Y
24[63,93,94]	2001	CA	7	M	Unknown/ Hispanic	Unknown	~45 (s)	Y
25[93]	2002	CA	64	M	Unknown/ Hispanic	Unknown, occupational soil exposure	~8 (s)	Y
26[95]	2002	FL	40	M	Unknown/ Unknown	Alcohol and drug abuse	~10 (h)	Y
27[96]	2002	NY	72	F	Unknown/ Unknown	Healthy, soil exposure	~27 (s)	N
28[93,94]	2003	CA	7	M	Unknown/ Hispanic	Steroid therapy	3 (h) from final hospitalization	Y
29[93]	2005	CA	19	M	Unknown/ Hispanic	Former drug abuse	8 (h)	Y
30[93]	2005	CA	12	M	Unknown/ Hispanic	Unknown, soil exposure	120 (h)	Y
31[93]	2007	CA	35	M	Unknown/ Hispanic	Unknown	Unknown	N
32[97]	2007	KY	2	M	Unknown/ Unknown	Healthy	~62 (h)	N
33[93,98]	2007	CA	43	M	White/ Unknown	Pneumonia, idiopathic thrombo-cytopenic purpura, occupational soil exposure	~117 (s)	Y
34[99]	2007	Unknown	Unknown	Unknown	Unknown/ Unknown	Multiple visceral organ transplant	Unknown	Y
35[93]	2007	CA	72	M	Pacific Islander/ Unknown	Unknown, gardening	7 (h)	Y

36[93]	2008	CA	1.6	M	Unknown/ Hispanic	Unknown	35 (h)	Y
37[18]	2009	MS	4	M	Unknown/ Unknown	Influenza A, water & soil exposure	~12 (s)	Y
38[18]	2009	MS	31	F	Unknown/ Unknown	Kidney transplant	~55 d (s)	Y
39[18]	2009	MS	27	M	Unknown/ Unknown	Kidney transplant	~139 (s)	N

*Clinical course may mean from symptom onset until death or resolution of illness (s) or from presentation/hospitalization until death or resolution of illness (h).
TB = Tuberculosis; HIV = Human Immunodeficiency Virus; IVDA = intravenous drug abuse

Table 5: Cutaneous acanthamoebiasis

Case (Ref)	Yr	State	Age (yr)	Sex	Race/ Ethnicity	Health Status	Clinical Course in Days	Died
1[6,22,39,100]	1989	NY	38	M	Unknown/ Unknown	HIV, pneumonia	>21 (s)	Y
2[22,23,39,44]	1990	IL	60	M	Unknown/ Hispanic	AIDS, PCP	~40 (s)	Y
3[22,39,40]	1991	CA	30	M	Unknown/ Unknown	AIDS, PCP, chronic sinusitis	~77 (s)	Y
4[39,101]	1991	VA	42	M	Unknown/ Unknown	AIDS	42 (s)	Y
5[22,34,102]	1992	NY	31	M	Unknown/ Unknown	ESRD, Kidney transplant, diabetes	~420 (s), con't treatment after lesions resolved	N
6[22,103]	1994	DC	44	M	Unknown/ Unknown	AIDS, progressive myelitis, peripheral neuropathy, water exposure	~450 (s)	Y
7[22,40,104]	1995 ?	CA	38	M	White/ Unknown	AIDS, PCP, chronic sinusitis, water exposure	Unknown	Y
8[22,105]	1996	CA	27	F	Unknown / Hispanic	AIDS	~240 (s)	Y
9[22,106]	1997 ?	MI	44	M	Unknown/ Unknown	AIDS, TB	~180 (s)	Y
10[22,106]	1997 ?	MI	38	M	Unknown/ Unknown	AIDS	~62 (s)	Y
11[107]	2003	MD	51	F	Unknown/ Unknown	AIDS, PCP	~31 (s)	N
12[108]	2004	CA	52	F	Unknown/ Unknown	Lung transplant, immune-Suppressants, creek as drinking water source	~150 (h)	N

*Clinical course may mean from symptom onset until death or resolution of illness(s) or from presentation/hospitalization until death or resolution of illness (h).
AIDS = Acquired Immune Deficiency Syndrome; HIV = Human Immunodeficiency Virus; PCP = *Pneumocystis carinii* pneumonia, ESRD = end-stage renal disease; TB = Tuberculosis

Table 6: *Acanthamoeba* Rhinosinusitis

Case (Ref)	Yr	State	Age (yr)	Sex	Race/ Ethnicity	Health Status	Clinical Course in Days	Died
1[53]	1994	Unk	45	M	Unknown/ Unknown	HIV, chronic sinusitis	Unknown	N
2[109]	2005	FL	49	F	Unknown/ Unknown	Bilateral lung transplant, immuno-suppressants, steroids	Unknown	N

*Clinical course may mean from symptom onset until death or resolution of illness (s) or from presentation/hospitalization until death or resolution of illness (h).
HIV = Human Immunodeficiency Virus

Table 7: *Acanthamoeba* osteomyelitis

Case (Ref)	Yr	State	Age (yr)	Sex	Race/ \Ethnicity	Health Status	Clinical Course in Days	Died
1[110]	1979	PA	32	F	White/ Unknown	Suspected diabetes	Unknown	N

*Clinical course may mean from symptom onset until death or resolution of illness (s) or from presentation/hospitalization until death or resolution of illness (h).

Appendix B: Case Report Form

Date of Report: _____

Demographics

Patient's Last Name	First	M.I.	Age	Gender: ☐Male ☐Female ☐Unknown

Ethnicity:	Race:	
☐Hispanic ☐Unknown ☐Non-Hispanic	☐White ☐Asian/Pacific Islander ☐Unknown ☐Black ☐American Indian ☐Other _____	County and State of Residence: _____ County and State of Treatment: _____

Immigrant? ☐ Yes ☐ No ☐ Unknown Country of origin: _____

Length of time since immigrated: _____

Occupation: _____

Exposure History

County/State of Suspected Exposure: _____ / _____ Number of persons exposed (if known): ____

Source of possible exposure, if known: *(please check all that apply and provide best estimates of dates)*

Water Exposures ☐ Yes ☐ No ☐ Unknown *If "**yes**", please fill out section on right*	**Type:**	**Date(s):**	**Type:**	**Date(s):**	**Type:**	**Date(s):**
	☐ Canal	_____	☐ Private Club Pool	_____	☐ Community Pool	_____
	☐ Lake	_____	☐ Private Home Pool	_____	☐ Apartment Pool	_____
	☐ Pond	_____	☐ Fill-and-Drain Pool	_____	☐ Fountain	_____
	☐ Ocean	_____	☐ Hotel Pool	_____	☐ Water park	_____
	☐ River/Stream	_____	☐ Spring (hot/cold)	_____		
	☐ Well	_____	☐ Spa/hot tub/whirlpool	_____		
	☐ Other, specify _____		Date(s): _____			

Water Activities ☐ *Yes* ☐ *No* ☐ *Unknown* *If "**yes**", please fill out specifics on right*		Yes	No	Unknown		Yes	No	Unknown
	Diving into water	☐	☐	☐	Snorkeling/scuba diving	☐	☐	☐
	Inhaled water	☐	☐	☐	Swimming	☐	☐	☐
	Jumped into water	☐	☐	☐	Water sports (skiing etc.)	☐	☐	☐
	Swallowed water	☐	☐	☐	Wore nose clip or plugged nose when jumping/diving	☐	☐	☐
	Splashed water	☐	☐	☐	Other, specify _____			

Soil Exposures ☐ Yes ☐ No ☐ Unknown *If "**yes**", please fill out specifics on right*	**Type:**	**Date(s):**
	☐ Gardening	_____
	☐ Composting	_____
	☐ Farm/Ranch	_____
	☐ Other, specify: _____	Date(s): _____

Occupational Exposures ☐ Yes ☐ No ☐ Unknown *If "**yes**", please fill out specifics on right*	
	☐ Farmer/rancher
	☐ Firefighter
	☐ Lifeguard/pool attendant
	☐ Other, specify: _____

Route of Entry if known: (please check all that apply)

☐ Inhalation ☐ Ingestion ☐ Contact ☐ Via Wound ☐ Other, specify: _____

If Water Source , Please List Source Characteristics:

Name of Water Exposure: _____ Geospatial Coordinates: _____ Thermally Polluted: Y / N

Size of Body Water: □ < 10 acres □ 10-100 acres □ >100 acres □ Unknown

Water Turbidity: □ Clear □ Cloudy □ Murky □ Unknown

Water level: □ Low □ High □ Normal □ Flood Stage □ Unknown

Flow Rate: □ Slow □ Normal □ Fast □ Unknown

Ambient Air Temperature: ___ F/C Water Temperature: ___F/C Depth: _____

Travel History last 2 years: ☐ Yes ☐ No ☐ Unknown If yes, please specify in table below:

Locations	Dates (from – to)

Past Medical History:

Please check all conditions/symptoms that patient has currently or has had within past 2 years:

Treatment/drugs:

- ☐ Excessive antibiotic use (specify in Provider comments)
- ☐ Illegal drug use, specify:_____
- ☐ Immunosuppressants
- ☐ Radiation therapy
- ☐ Steroid use

HIV/AIDS:

HIV	☐ Yes	☐ No	☐ Unknown
AIDS	☐ Yes	☐ No	☐ Unknown
On Antiretrovirals	☐ Yes	☐ No	☐ Unknown

Other Immunocompromised Conditions

- ☐ Alcohol misuse ☐ Diabetes mellitus
- ☐ G6PD deficiency ☐ Liver cirrhosis
- ☐ Malnourishment ☐ Pregnancy (recent)
- ☐ Renal failure ☐ Lymphoproliferative disease
- ☐ Systemic Lupus Erythematosus (SLE)
- ☐ Cancer, specify:_____
- ☐ Other hematologic disease, specify: _____
- ☐ Other autoimmune disease, specify: _____
- ☐ Organ transplant, specify: _____

ENT/Respiratory:

- ☐ Otitis
- ☐ Rhinitis
- ☐ Broken Nose
- ☐ Deviated septum
- ☐ Tuberculosis
- ☐ Other lung disease, specify: ____
- ☐ Sinusitis
- ☐ Epistaxis
- ☐ Nasal Surgery
- ☐ Pharyngitis
- ☐ Pneumonitis

Other Conditions:

- ☐ Dermatitis
- ☐ Skin infections
- ☐ Eye infection
- ☐ Other, specify: _____
- ☐ Injury, specify: _____

Current Illness

Date of Illness onset: _____ Duration of illness: (in days) ____

Was patient admitted to hospital for current illness? ☐ Yes ☐ No ☐ Unknown

If Yes, Date of **most recent** hospitalization: _____ Duration of most recent hospitalization (in days): _____

Hospital Name:_____ City:_____ State: _____

Physician Name 1:_____ E-mail (if avail): _____ Phone: _____

Physician Name 2:_____ E-mail (if avail): _____ Phone: _____

Other Recent Hospitalizations: ☐Yes ☐ No☐Unknown

Dates (from- to)	Diagnosis

History of Present Illness:

Please provide a brief description of the patient's clinical course, prior to hospitalization:

Signs/Symptoms on Presentation (*most recent* hospitalization):

Vital Signs:

Temperature: _____ F / C P: _____ bpm R= _____breaths/min BP: _____mmHg

General:

	Duration (days)		Duration (days)
☐ Fever	_____	☐ Myalgia	_____
☐ Nausea	_____	☐ Back Pain	_____
☐ Vomiting	_____	☐ Cough	_____
☐ Diarrhea	_____	☐ Shortness of breath	_____
☐ Weight loss	_____	☐ Sinus problems	_____
☐ Anorexia	_____	☐ Abnormal reflexes	_____
☐ Headache	_____	☐ Disorientation	_____
☐ Stiff neck	_____	☐ Lethargy/fatigue	_____
☐ Other general symptom/sign, specify:_____			_____

Visual

	Duration (days)
☐ Blurred vision	_____
☐ Diplopia	_____
☐ Photophobia	_____
☐ Other visual changes, specify: _____	_____

Neurologic:

	Duration (days)		Duration (days)		Duration (days)
☐ Altered mental status	_____	☐ Dysphagia	_____	☐ Weakness	_____
☐ Aphasia	_____	☐ Facial numbness/Parathesia	_____	☐ Hemiparesis/Hemiplegia	_____
☐ Ataxia	_____	☐ Hallucinations	_____	☐ Altered sense of taste	_____
☐ Behavioral changes	_____	☐ Combativeness	_____	☐ Altered sense of smell	_____
☐ Coma	_____	☐ Hyperreflexia	_____	☐ Decerebrate posturing	_____
☐ Confusion	_____	☐ Loss of balance	_____	☐ Decorticate posturing	_____
☐ Cranial nerve VI deficit	_____	☐ Numbness	_____	☐ Fixed, nonreactive pupils	_____
☐ Cranial nerve VII deficit	_____	☐ Seizures	_____	☐ Dilated pupils	_____
☐ Cranial nerve XII deficit	_____	☐ Upgoing toes	_____	☐ Nystagmus	_____

☐ Other cranial nerve deficit, specify: _____ Duration: _____

☐ Other neurologic deficit, specify: _____ Duration: _____

Skin Lesions: ☐ Yes ☐ No ☐ Unknown *If yes, please specify in table below*:

Lesion type	Anatomic location	Size	Number	Duration (days)
Ulcers				
Plaques				
Erythematous nodules				
Other				

Other Symptoms/Signs:

☐ Other, specify:_____

Signs/Symptoms developed while in hospital:

General:

- ☐ Fever
- ☐ Myalgia
- ☐ Nausea
- ☐ Back Pain
- ☐ Vomiting
- ☐ Cough
- ☐ Diarrhea
- ☐ Shortness of breath
- ☐ Weight loss
- ☐ Sinus problems
- ☐ Anorexia
- ☐ Abnormal Reflexes
- ☐ Headache
- ☐ Disorientation
- ☐ Stiff neck
- ☐ Lethargy/fatigue
- ☐ Other general symptom/sign, specify:_____

Visual

- ☐ Blurred vision
- ☐ Diplopia
- ☐ Photophobia
- ☐ Other visual changes, specify:_____

Neurologic:

- ☐ Altered mental status
- ☐ Aphasia
- ☐ Ataxia
- ☐ Behavioral changes
- ☐ Coma
- ☐ Combativeness
- ☐ Confusion
- ☐ Cranial nerve VI deficit
- ☐ Cranial nerve VII deficit
- ☐ Cranial nerve XII deficit
- ☐ Other Cranial nerve deficit, specify: _____
- ☐ Dysphagia
- ☐ Facial numbness/Parathesia
- ☐ Hallucinations
- ☐ Hemiparesis/Hemiplegia
- ☐ Hyperreflexia
- ☐ Loss of balance
- ☐ Numbness
- ☐ Seizures
- ☐ Upgoing toes
- ☐ Weakness
- ☐ Other neurologic deficit, specify: _____
- ☐ Altered sense of taste
- ☐ Altered sense of smell
- ☐ Decerebrate posturing
- ☐ Decorticate posturing
- ☐ Fixed, nonreactive pupils
- ☐ Dilated pupils
- ☐ Nystagmus

Skin Lesions: ☐ Yes ☐ No *If yes, please specify in table below:*

Lesion type	Anatomic location	Size	Number
Ulcers			
Plaques			
Erythematous nodules			
Other			

Other Symptoms/Signs:

☐ Other, specify:_____

Diagnostic Tests: Note please provide dates when possible. If date not available, provide hospital day (i.e. CSF tap on Hosp. Day 2)

LABORATORY TESTING

CSF	Date_____	Date_____	Date_____
	Results	**Results**	**Results**
Opening pressure (mmH2O)			
WBC count (per mm^3)			
RBC count (per mm^3)			
Neutrophil %			
Monocyte %			
Lymphocyte %			
Bands %			
Eosinophil %			
Protein (mg/100ml)			
Glucose (mg/100ml)			
Cytokines			
CSF Culture: *			
CSF PCR: *			
CSF latex agglutination: *			
CSF mount: *Please indicate preparation type and findings, if any*	☐ Centrifuged ☐ Stained ☐ Wet Amebae present? Y☐ N☐	☐ Centrifuged ☐ Stained ☐ Wet Amebae present? Y☐ N☐	☐ Centrifuged ☐ Stained ☐ Wet Amebae present? Y☐ N☐

*** Please provide results for all bacteria, viral and/or parasitic testing.**

Presenting Lab Values: Date:____

	Results
RBC count (per mm^3)	
Hematocrit %	
WBC count (per mm^3)	
Neutrophil %	
Lymphocyte %	
Monocyte %	
Eosinophil %	
Bands %	
CD4 count (per mm^3)	
Protein (mg/100ml)	
Sodium (mEq/L)	
Potassium (mEq/L)	
Chloride (mEq/L)	
Bicarbonate (mEq/L)	
BUN (mg/100ml)	
Creatinine (mg/100ml)	
Glucose (mg/100ml)	

Serology:

Date	**Result**

Cultures for Free Living Amebae:

Source	**Date**	**Result**
☐ Blood		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba
☐ Skin		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba
☐ Brain		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba
☐ Abscess		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba
☐ Other, specify: _____		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba

PCR for Free Living Amebae:

Source	**Date**	**Result**
☐ Blood		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba
☐ Skin		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba
☐ Brain		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba
☐ Abscess		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba
☐ Other, specify: _____		☐ Negative ☐ + Balamuthia ☐ + Naegleria ☐ + Acanthamoeba

HISTOPATHOLOGY

Brain biopsy: ☐ Yes ☐ No ☐ Unknown

	Date:	Date:
Location		
Timing	☐ Antemortem ☐ Postmortem ☐ Unknown	☐ Antemortem ☐ Postmortem ☐ Unknown
Results (check all that apply)	☐ Amebic trophozoites	☐ Amebic trophozoites
	☐ Amebic cysts	☐ Amebic cysts
	☐ Ameba, not specified	☐ Ameba, not specified
	☐ Hemorrhage ☐ Necrosis	☐ Hemorrhage ☐ Necrosis
	☐ Encephalomalacia ☐ Edema	☐ Encephalomalacia ☐ Edema
	☐ Abscess ☐ Vasculitis	☐ Abscess ☐ Vasculitis
	☐ Perivascular Inflammation	☐ Perivascular Inflammation
	☐ Thrombosis	☐ Thrombosis
	☐ Neovascularization	☐ Neovascularization
	☐ Neutrophillic inflammation / infiltrate	☐ Neutrophillic inflammation / infiltrate
	☐ Lymphocytic inflammation / infiltrate	☐ Lymphocytic inflammation / infiltrate
	☐ Granulomatous inflammation	☐ Granulomatous inflammation
	☐ Granuloma	☐ Granuloma
	☐ Meningitis ☐ Encephalitis	☐ Meningitis ☐ Encephalitis
	☐ Meningoencephalitis	☐ Meningoencephalitis
Other Results/ Comments		

Skin biopsy: ☐ Yes ☐ No ☐ Unknown

	Date:	Date:
Location		
Timing	☐ Antemortem ☐ Postmortem ☐ Unknown	☐ Antemortem ☐ Postmortem ☐ Unknown
Results	☐ No amebae seen ☐ Amebic trophozoites ☐ Amebic cysts ☐ Amebae, not specified	☐ No amebae seen ☐ Amebic trophozoites ☐ Amebic cysts ☐ Amebae, not specified
Other Results/ Comments		

Sinus biopsy: ☐ Yes ☐ No ☐ Unknown

	Date:	Date:
Location		
Timing	☐ Antemortem ☐ Postmortem ☐ Unknown	☐ Antemortem ☐ Postmortem ☐ Unknown
Results	☐ No amebae seen ☐ Amebic trophozoites ☐ Amebic cysts ☐ Amebae, not specified	☐ No amebae seen ☐ Amebic trophozoites ☐ Amebic cysts ☐ Amebae, not specified
Other Results/ Comments		

Other biopsy results:

DIAGNOSTIC IMAGING

CT: Date of First CT: _____

Lesion location: (please check all that apply)

- ☐ Basal Ganglia
- ☐ Brainstem
- ☐ Right Cerebellum
- ☐ Left Cerebellum
- ☐ Left Frontal
- ☐ Right Frontal
- ☐ Left Occipital
- ☐ Right Occipital
- ☐ Left Parietal
- ☐ Right Parietal
- ☐ Pons
- ☐ Spinal Cord
- ☐ Left Temporal
- ☐ Right Temporal
- ☐ Thalamus
- ☐ Other, specify:_____

Lesion: (please check all that apply)

- ☐ Abscess
- ☐ Edema
- ☐ Erosion
- ☐ Hemorrhage
- ☐ Herniation
- ☐ Hyperdense
- ☐ Hypodense
- ☐ Infarcts
- ☐ Mass
- ☐ Multifocal lesions
- ☐ Enhancing
- ☐ Ring enhancing
- ☐ Sinusitis
- ☐ Ventriculomegaly
- ☐ Other, specify: _____

Additional Description, if needed:

_____

Please list dates of subsequent CT scans and changes noted:

Date	Findings

MRI: Date of First MRI: _____

Lesion location: (please check all that apply)

- ☐ Basal Ganglia
- ☐ Brainstem
- ☐ Right Cerebellum
- ☐ Left Cerebellum
- ☐ Left Frontal
- ☐ Right Frontal
- ☐ Left Occipital
- ☐ Right Occipital
- ☐ Left Parietal
- ☐ Right Parietal
- ☐ Pons
- ☐ Spinal Cord
- ☐ Left Temporal
- ☐ Right Temporal
- ☐ Thalamus
- ☐ Other, specify:_____

Lesion: (please check all that apply)

- ☐ Abscess
- ☐ Edema
- ☐ Erosion
- ☐ Hemorrhage
- ☐ Herniation
- ☐ Hyperdense
- ☐ Hypodense
- ☐ Infarcts
- ☐ Mass
- ☐ Multifocal lesions
- ☐ Enhancing
- ☐ Ring enhancing
- ☐ Sinusitis
- ☐ Ventriculomegaly
- ☐ Other, specify: _____

Additional Description, if needed:

_____

Please list dates of subsequent MRI scans and changes noted:

Date	Findings

Diagnosis:

Admitting diagnosis: Date of Diagnosis: _____

☐ Encephalitis ☐ Meningoencephalitis
☐ Meningitis ☐ Other, Specify _____

Final diagnosis: Date of Diagnosis: _____

☐ GAE (Acanthamoeba spp.) ☐ GAE (Balamuthia mandillaris) ☐ PAM (Naegleria fowleri)
☐ Disseminated Acanthamoebiasis ☐ Disseminated Balamuthiasis ☐ Other, specify: _____
☐ Acanthamoeba rhinosinusitis ☐ Balamuthia rhinosinusitis
☐ Cutaneous acanthamoebiasis ☐ Cutaneous balamuthiasis

Diagnosis Method:

Indirect Immunofluorescence (IIF) ☐	Antemortem ☐	Postmortem ☐	Unknown ☐
PCR ☐	Antemortem ☐	Postmortem ☐	Unknown ☐
Histopathology ☐	Antemortem ☐	Postmortem ☐	Unknown ☐
CSF Wet Mount ☐	Antemortem ☐	Postmortem ☐	Unknown ☐
Culture ☐	Antemortem ☐	Postmortem ☐	Unknown ☐
Other, Specify:	Antemortem ☐	Postmortem ☐	Unknown ☐

Treatment:

Surgical resection: ☐ Yes ☐ No

Medications: (please check all that apply)

☐ Acyclovir	☐ Ethambutol	☐ Rifampin
☐ Albendazole	☐ Fluconazole	☐ Steroid, specify_____
☐ Amikacin	☐ Flucytosine	☐ Streptomycin
☐ Amphotericin B	☐ Isoniazid	☐ Sulfonamide, specify_____
☐ Amphotericin B lipid	☐ Itraconazole	☐ Sulfadiazine
☐ Amphotericin B liposomal	☐ Ketoconazole	☐ Topical Chlorhexidine
☐ Azithromycin	☐ Mannitol	☐ Trimethoprim/sulfa
☐ Ceftriaxone	☐ Metronidazole	☐ Voriconazole
☐ Ciprofloxacin	☐ Miconazole	☐ Other, specify_____
☐ Chloramphenicol	☐ Miltefosine	☐ Other, specify_____
☐ Clarithromycin	☐ Ornidazole	☐ Other, specify_____
☐ Co-trimoxazole	☐ Pentamidine	☐ Other, specify_____
☐ Dexamethasone	☐ Pyrimethamine	☐ Other, specify_____

If you checked any of the medications listed above, please list below with the start and stop dates, doses, frequencies, and routes of administration. If an individual drug was given using multiple doses, over multiple dates, and/or given by multiple routes, please indicate this in the rows below.

Medication	Start date:	Stop date:	Dose and Frequency	Route of Administration

Other therapies: (please check all that apply)

	Start date:	Stop date:
☐ IV fluids		
☐ Total Parenteral Nutrition (TPN)		
☐ Dialysis for renal failure		
☐ Other, specify_____		
☐ Other, specify_____		
☐ Other, specify_____		

Outcome:

Survived? Yes ☐ No ☐ Unknown ☐

If survived: Residual neurologic deficits? Yes ☐ No ☐ Unknown ☐

If Yes, Please describe neurologic deficits: _____

Date of discharge: _____ OR Date of death: _____

If died: Cause of death:

☐ Brain death ☐ Removed life support
☐ Cardiorespiratory failure ☐ Other, specify:_____
☐ Herniation

If died: Organs ☐ Yes ☐ No transplanted?

If yes, which ones:_____

Please provide a brief description of the patient's clinical course, complications, and any additional comments:

CDC USE ONLY:

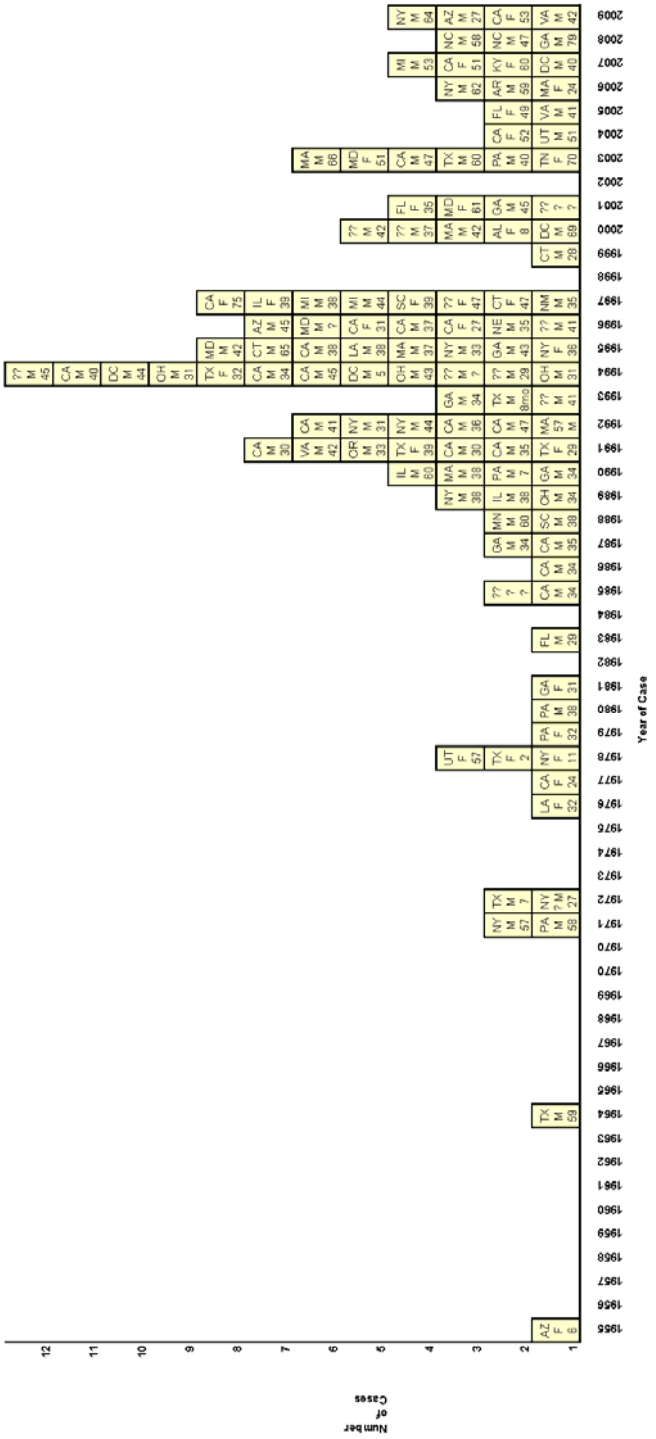
1st DASH #	
2nd DASH #	
3rd DASH #	
4th DASH #	
5th DASH #	
List additional DASH #s:	

Case report citation 1	
Case report citation 2	
List additional case citations	

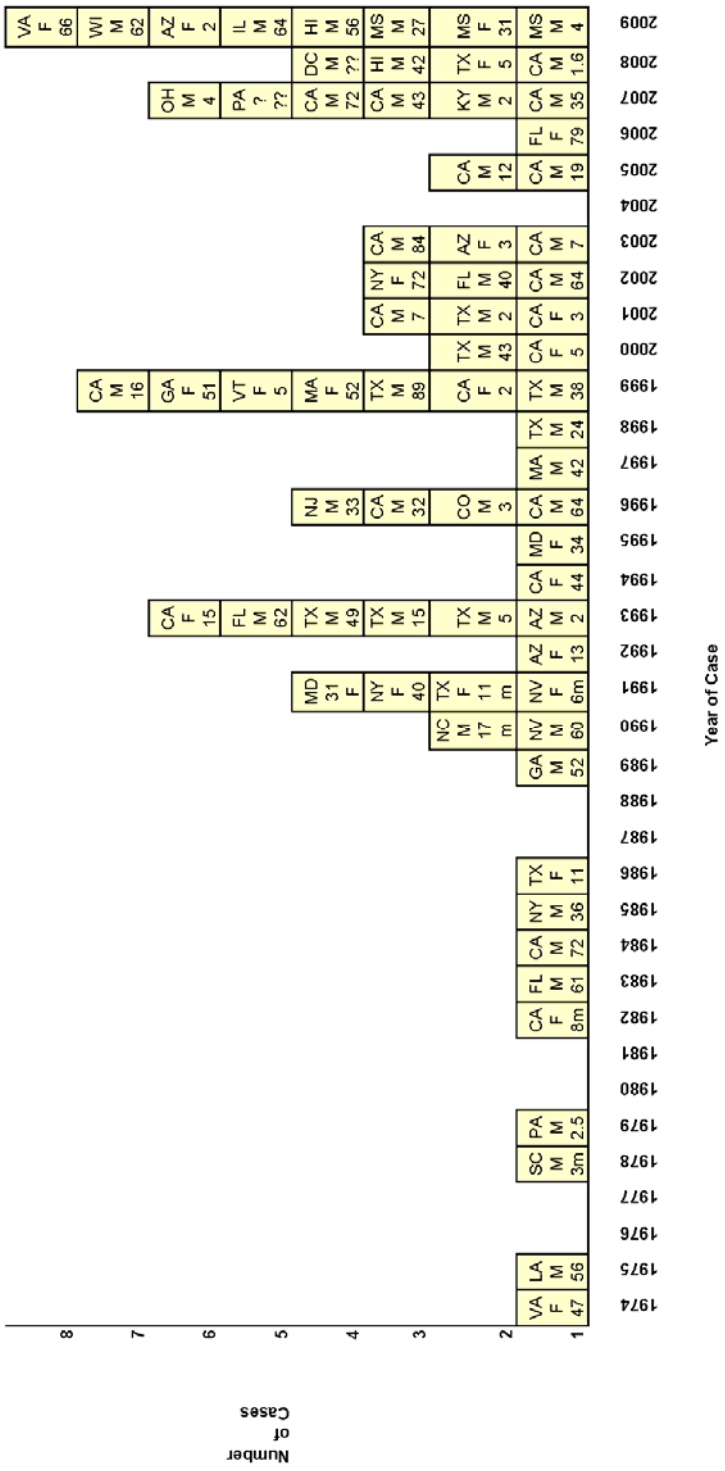
Calculated durations:
Incubation period (days): _____
Illness Onset to Admission (days): _____
Illness Onset to Death (days): _____
Exposure to Death (days): _____
Clinical Stage at presentation: _____

Appendix C: Epidemic Curves for *Acanthamoeba* and *Balamuthia* Cases

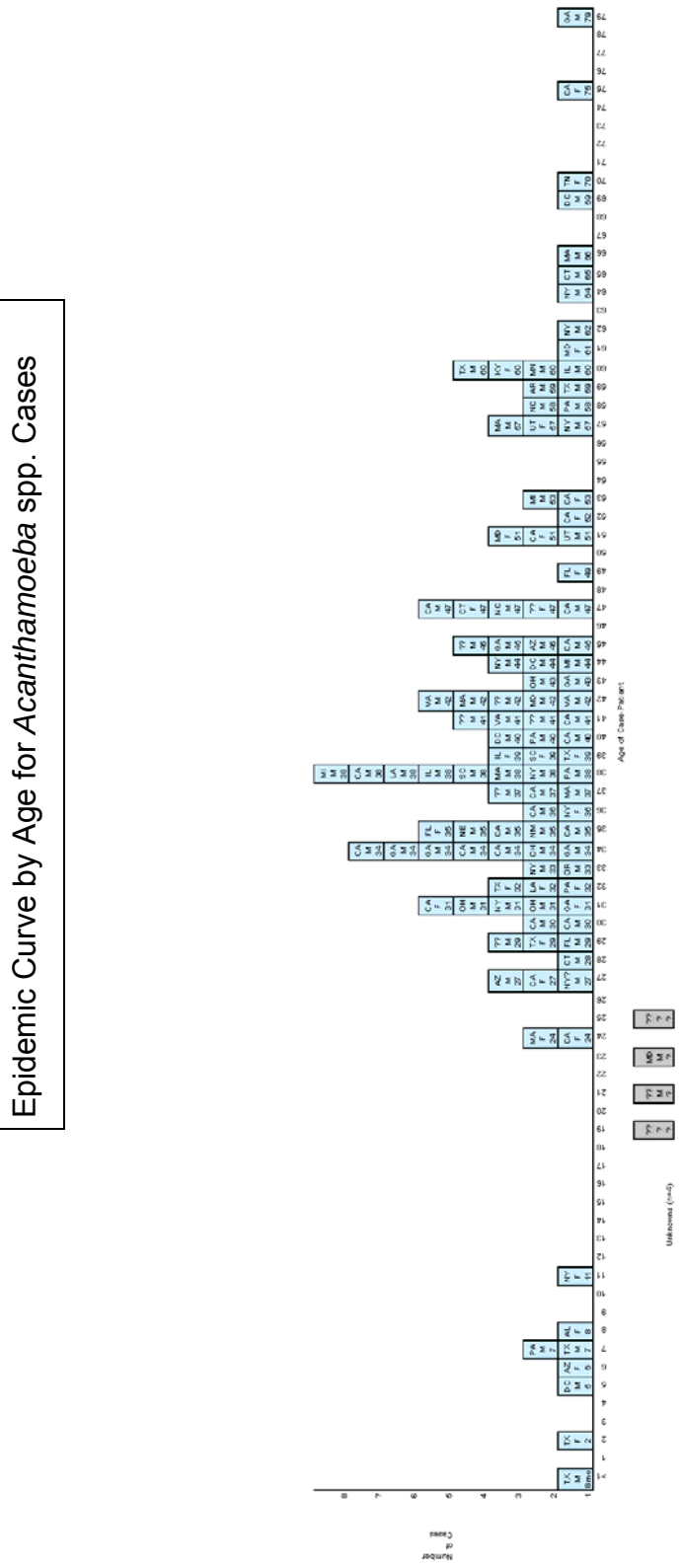
Epidemic Curve by Year of Report for *Acanthamoeba* spp. Cases



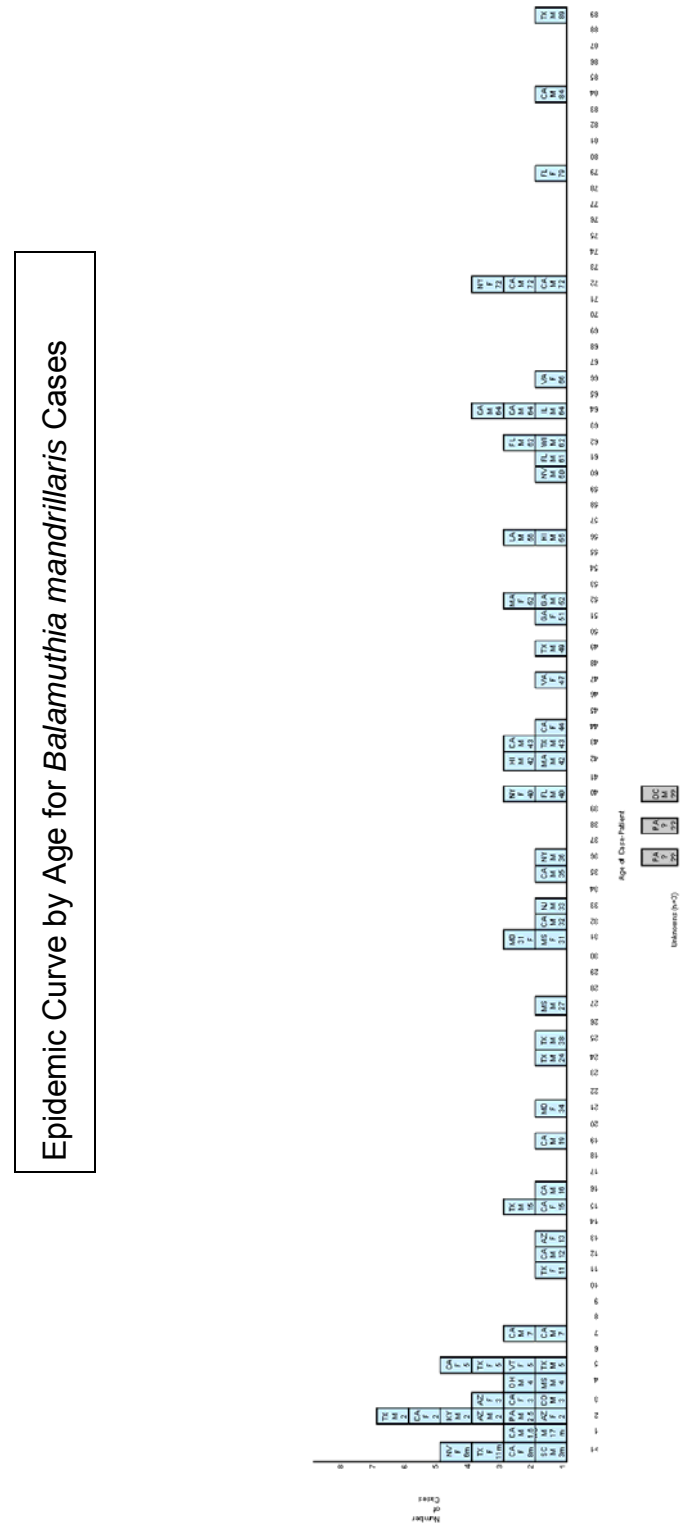
Epidemic Curve by Year of Report for *Balamuthia mandrillaris* Cases



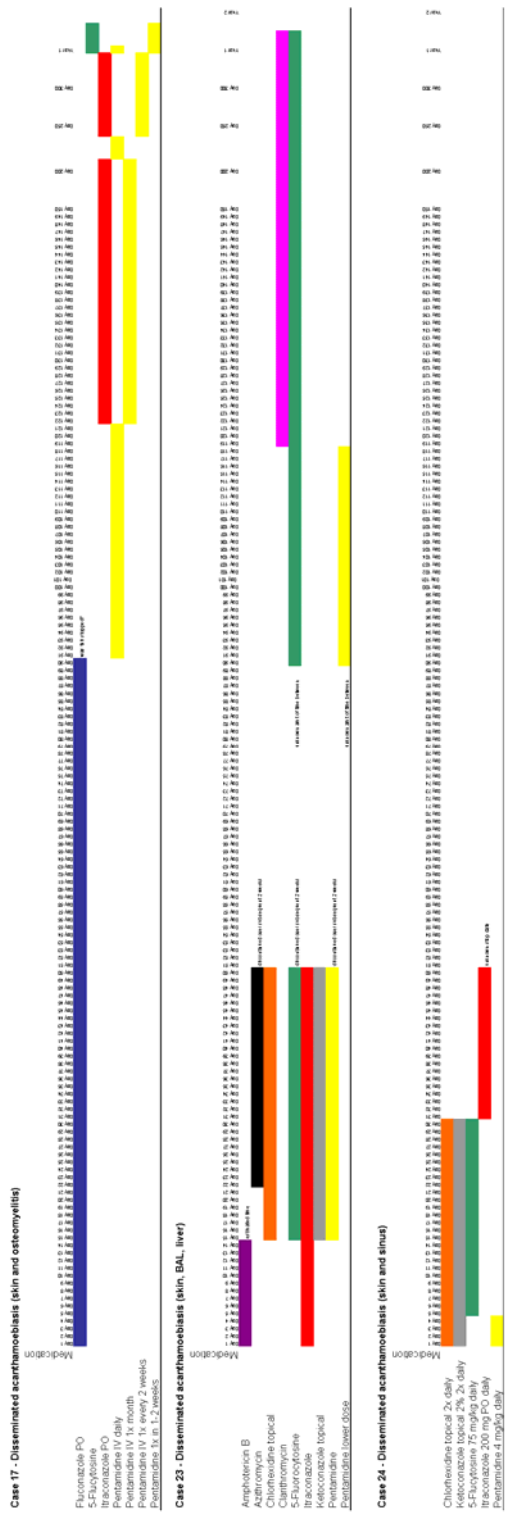
Epidemic Curve by Age for *Acanthamoeba* spp. Cases



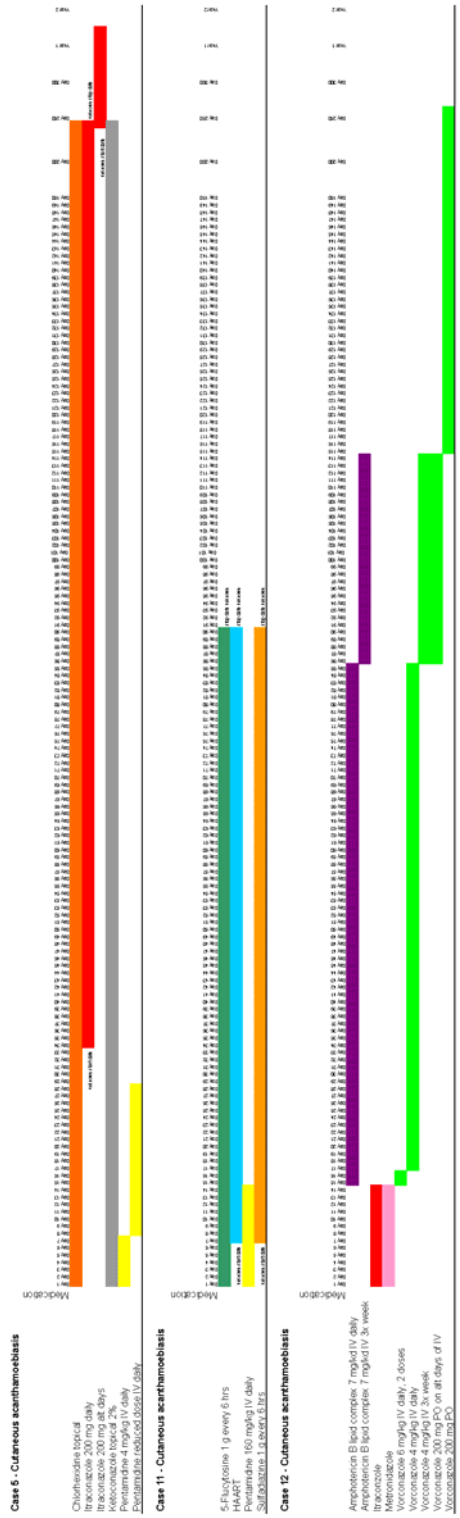
Epidemic Curve by Age for *Balamuthia mandrillaris* Cases



Appendix D: Treatment Regimens for Survivors



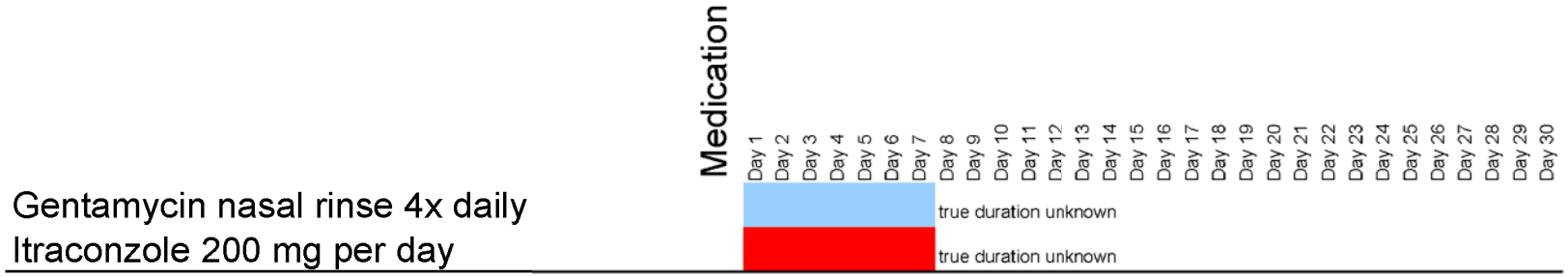
Survivors of disseminated acanthamoebiasis



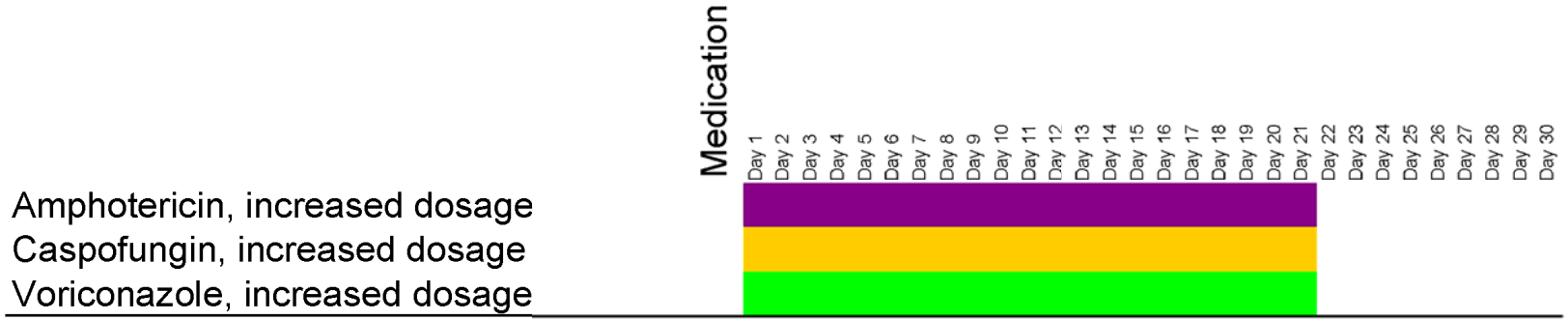
Survivors of cutaneous acanthamoebiasis

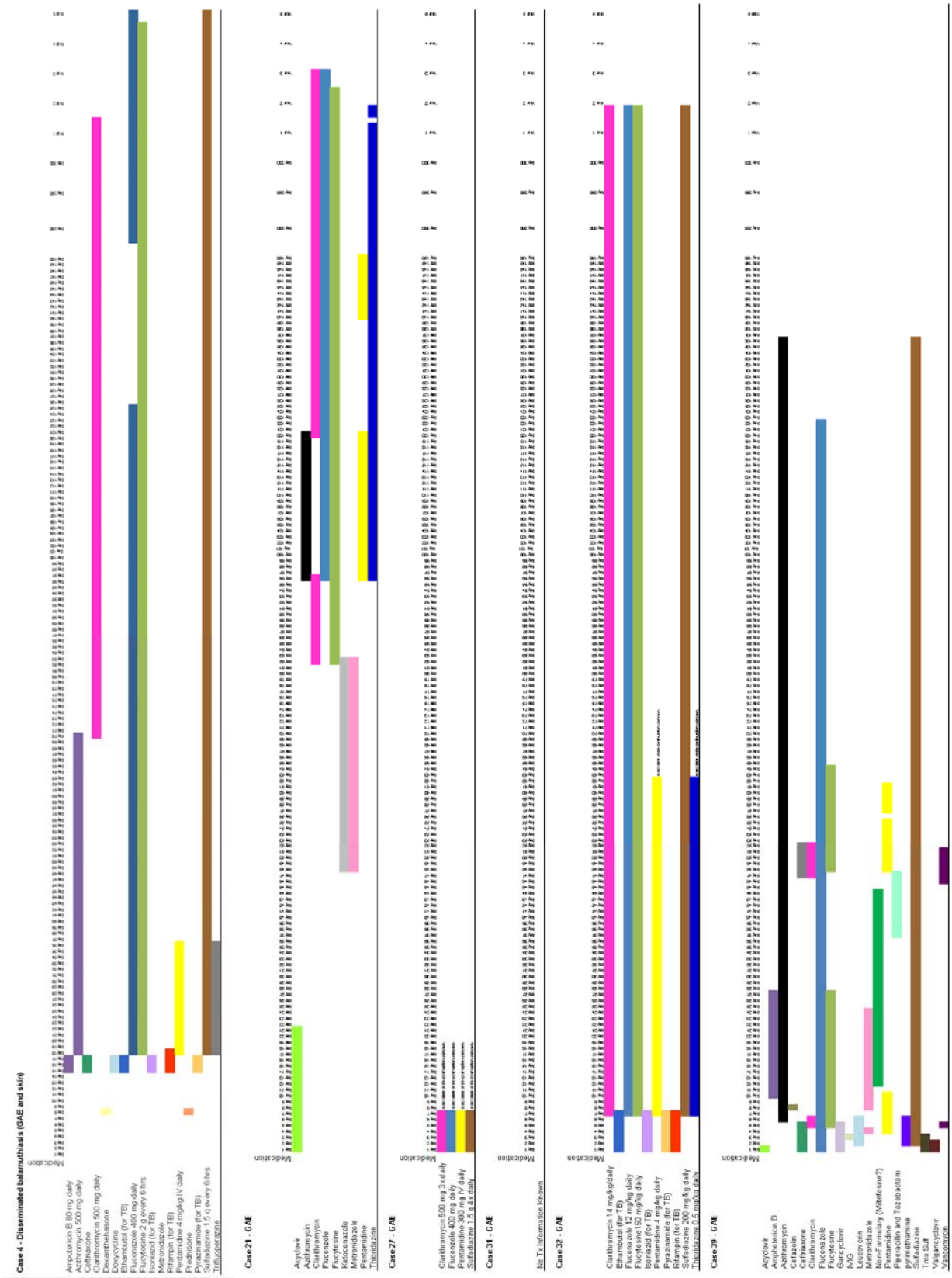
Survivors of *Acanthamoeba* rhinosinusitis

Case 1 - *Acanthamoeba* Rhinosinusitis



Case 2 - *Acanthamoeba* Rhinosinusitis





Survivor of disseminated balamuthiasis

Survivors of *Balamuthia* GAE