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Epidemiology of Free-Living Ameba Infections[1]

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ABSTRACT. Small free-living amebas belonging to the genera *Acanthamoeba* and *Naegleria* occur world-wide. They have been isolated from a variety of habitats including fresh water, thermal discharges of power plants, soil, sewage and also from the nose and throats of patients with respiratory illness as well as healthy persons. Although the true incidence of human infections with these amebas is not known, it is believed that as many as 200 cases of central nervous system infections due to these amebas have occurred worldwide. A majority (144) of these cases have been due to *Naegleria fowleri* which causes an acute, fulminating disease, primary amebic meningoencephalitis. The remaining 56 cases have been reported as due either to *Acanthamoeba* or some other free-living ameba which causes a subacute and/or chronic infection called granulomatous amebic encephalitis (GAE). *Acanthamoeba*, in addition to causing GAE, also causes nonfatal, but nevertheless painful, vision-threatening infections of the human cornea, *Acanthamoeba* keratitis. Infections due to *Acanthamoeba* have also been reported in a variety of animals. These observations, together with the fact that *Acanthamoeba* spp., *Naegleria fowleri*, and *Hartmannella* sp. can harbor pathogenic microorganisms such as *Legionella* and or mycobacteria indicate the public health importance of these amebas.

Key words. *Acanthamoeba*, *Acanthamoeba* keratitis, acquired immunodeficiency syndrome, contact lens, granulomatous amebic encephalitis, *Naegleria*, primary amebic meningoencephalitis.

SMALL free-living amebas belonging to the genera *Acanthamoeba* and *Naegleria* occur world-wide. They have been isolated from a variety of habitats. *Naegleria* spp., e.g., have been isolated from fresh water ponds and lakes, domestic water supply, thermal discharges of power plants, hot springs and spas, swimming pools, hydrotherapy pools, remedial pools, aquaria, soil, sewage and even nasal passages of healthy children [22, 30]. *Acanthamoeba* spp. also have been isolated from soil, fresh water, bottled mineral water, mushrooms and vegetables, brackish and sea water as well as ocean sediments, cooling towers of electric and nuclear power plants, physiotherapy pools and medicinal pools, swimming pools, heating, ventillating and air conditioning units, dialysis units, gastrointestinal washings, dental units, dust in air, sewage, and bacterial, fungal, and mammalian cell cultures. In humans they have been found in the nose and throat of patients with respiratory illness as well as from healthy persons, and in bronchial secretions, ear discharge, and stool samples of patients with diarrhea. More recently they have been isolated from hot tubs, contact lens-care solutions, and intrauterine contraceptive devices [2, 8, 12, 22, 45, 58, 64, 65, 75]. These amebas have also been implicated in humidifier fever, and an allergic hypersensitivity pneumonitis illness. Although the true incidence of human infections with these amebas is not known, it is believed that as many as 200 cases of central nervous system (CNS) infections due to these amebas have occurred world-wide. A majority of these cases (144) have been due to *Naegleria fowleri*. The remaining 56 cases have been reported as due either to *Acanthamoeba* or some other free-living ameba (Table 1). *Acanthamoeba*, in addition to causing CNS disease, is also known to cause nonfatal, but nevertheless painful, infections of the human cornea, *Acanthamoeba* keratitis.

Infections due to *N. fowleri*. At this time six species of *Naegleria* have been described in the literature. They are *N. andersoni, N. australiensis, N. fowleri, N. gruberi, N. jadini*, and *N. lovaniensis*. Although both *N. australiensis* and *N. fowleri* are known to be thermophilic and pathogenic to mice [22, 30, 45], only *N. fowleri* is thought to cause disease in humans. Most of the human isolates have been shown to be *N. fowleri* by various

[1] Based in part on a keynote address presented by G. S. Visvesvara at the Vth International Conference on the Biology and Pathogenicity of Free-Living Amoebae, Brussells, Belgium, August 7–11, 1989.

Table 1. Primary amebic meningoencephalitis (PAM) and granulomatous amebic encephalitis (GAE). (No. of cases world-wide as of January, 1990.)[a]

Country	PAM	GAE	Total
Australia	19	2	21
Barbados	0	1	1
Belgium	5	0	5
Brazil	5	0	5
China	0	2	2
Cuba	1	0	1
Czechoslovakia	18	0	18
Honduras	0	2	2
Hungary	1	0	1
India	9	3	12
Japan	0	2	2
Mexico	3	1	4
New Guinea	1	0	1
New Zealand	8	0	8
Nigeria	4	1	5
Panama	1	0	1
Peru	0	8	8
South Africa	0	1	1
Uganda	1	0	1
United Kingdom	3	0	3
United States	63	30	93
Venezuela	2	2	4
Zambia	0	1	1
	144	56	200

[a] Modified from Martinez, A. J. [44].

experimental studies, including antigenic makeup [21, 30, 45, 78], isoenzyme analysis [18, 19, 21, 50, 54], and/or restriction fragment length polymorphism of the mitochondrial or genomic DNA [20, 48]. All other species of *Naegleria* are nonpathogenic although *N. lovaniensis* and *N. andersoni* are known to grow at higher temperatures. *Naegleria fowleri* causes an acute hemorrhagic necrotizing meningoencephalitis called primary amebic meningoencephalitis (PAM), which almost always causes death within 7 to 10 days [45]. Most of these cases have occurred in children or young adults in good health with a history of swimming in or contact with fresh water before their illness. Since *N. fowleri* is thermophilic and proliferates extensively at higher temperatures (up to 45° C), most of the cases of PAM, especially in the United States, have occurred during summer months when the ambient temperature is high. The 1st case of PAM due to *N. fowleri* was reported from Australia in 1965, though at that time, it was described as being due to *Acanthamoeba* [28]. A retrospective study by St. Symmers [67], however, indicates that the 1st recorded case probably occurred in Ireland in 1909. As many as 144 cases of PAM due to *N. fowleri* (Table 1) have been reported from all over the world since then. Only four patients have been reported to have recovered from this disease [22].

Primary amebic meningoencephalitis in the United States. The 1st case of PAM in the United States was reported from Florida by Butt in 1966 [6], although a retrospective study revealed that the 1st case probably occurred in Virginia in 1939 [23]. Since then, 63 cases have been recorded at the Centers for Disease Control (CDC), primarily from the southern, northeast, and west coast states (Fig. 1). Of these 63 cases, 20 have occurred

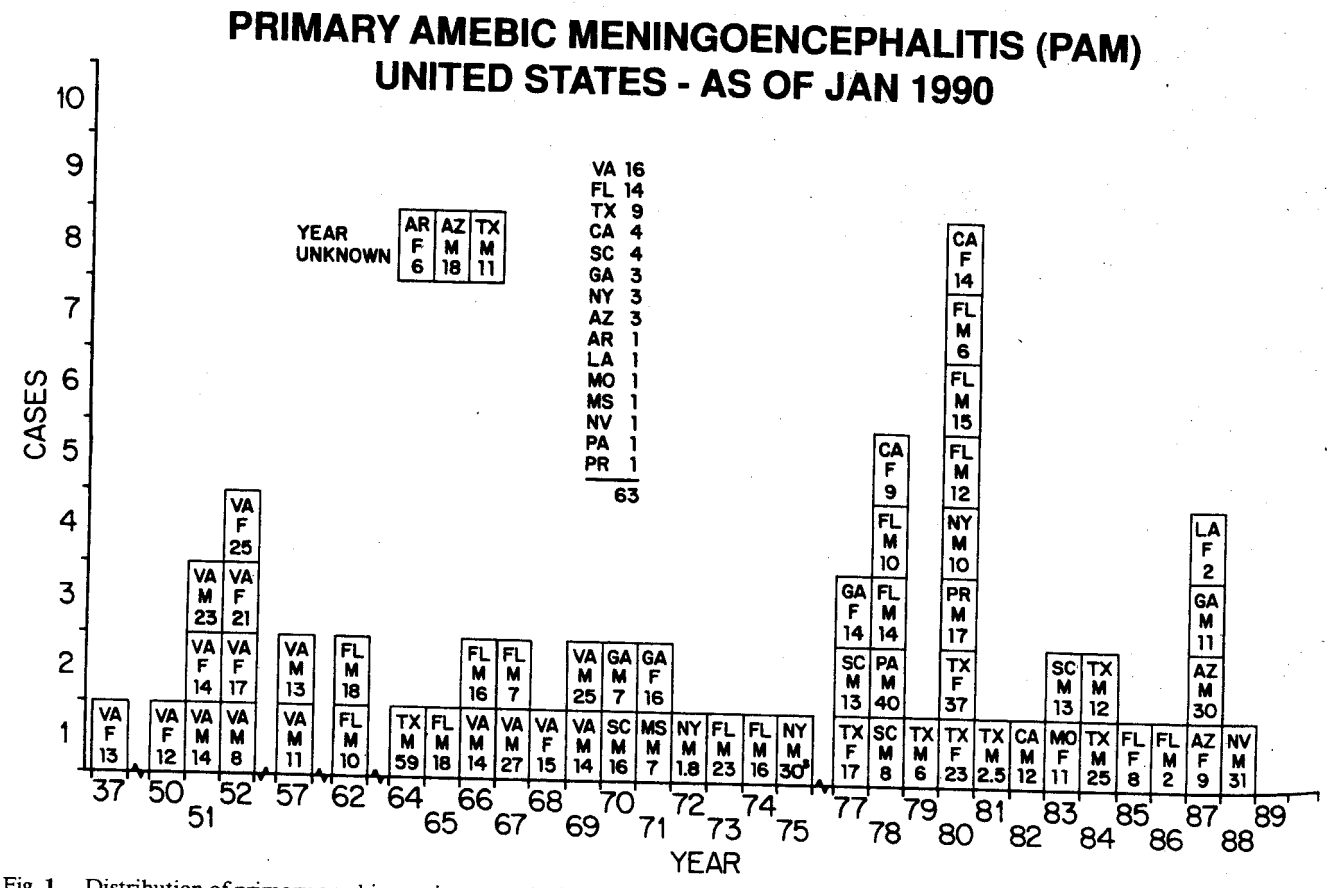

Fig. 1. Distribution of primary amebic meningoencephalitis cases in the United States, 1937 through January 1990. Each rectangle represents a case and includes information on the state in which it occurred and the sex (F = female; M = male) and age of the patient.

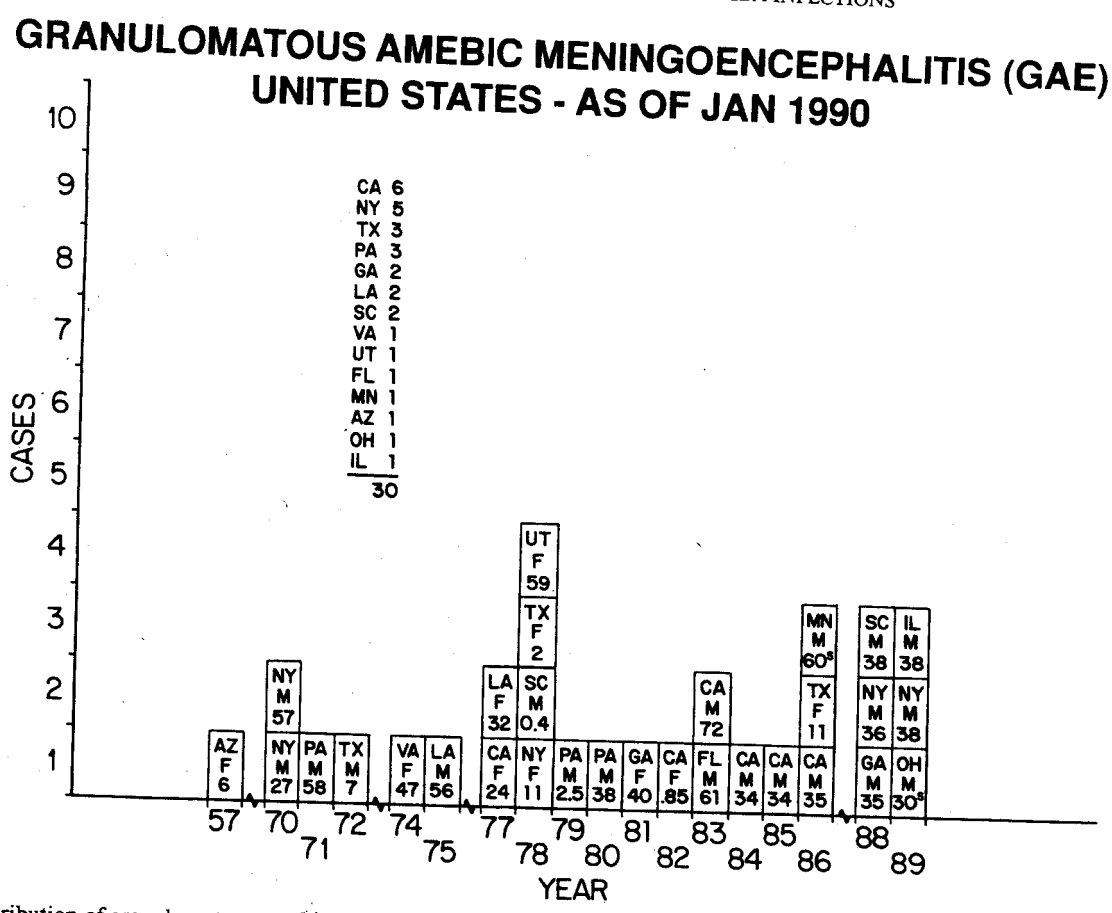

Fig. 2. Distribution of granulomatous amebic encephalitis cases in the United States, 1957 through January 1990. Each rectangle represents a case and includes information on the state in which it occurred and the sex (F = female; M = male) and age of the patient.

in females and 43 in males. As many as eight cases, the maximum number recorded for any year in the United States including Puerto Rico, occurred in 1980 [7]. Of these, 3 cases occurred in Florida, 2 in Texas, and 1 each in New York, California, and Puerto Rico. An epidemiologic study conducted at that time revealed that all patients had onset of illness in June, July, or August. All patients died, although amebas were identified in the cerebrospinal fluid (CSF) before death in four of the patients who received specific anti-*N. fowleri* chemotherapy with amphotericin B. The mean interval after onset of symptoms until death was 6.4 days, with a range from 2 to 12 days.

The average age of the 3 female patients was 24.6 yr, with a range of 14 to 37 yr, whereas the 5 male patients had an average age of 12 yr, with a range of 6 to 17 yr. The following signs and symptoms were recorded by the physicians at the initial visit of the patients: headache (8/8), nausea or vomiting (6/8), fever greater than 39° C (8/8), meningismus (6/8), anorexia (5/8), and other mental status changes such as stupor and agitation, lethargy and obtundation, or combativeness (3/8). The 1st lumbar puncture performed on each patient revealed the following: mean CSF leukocyte count 2,780 cells per mm³ (range 133 to 8,320 cells); mean percentage of polymorphonuclear cells 80% (range 57% to 99%); mean CSF protein concentration 413 mg/dl (range 84 to 1,210 mg/dl); and mean CSF glucose level 43 mg/dl (range 3 to 87 mg/dl).

A serologic confirmation of a diagnosis of PAM is not currently available. Most of the patients with *Naegleria* PAM have died within a very short time (5–10 days), before they have had sufficient time to produce detectable levels of antibody. Hence, previous attempts to determine antibody titers to *N. fowleri* with the use of a sensitive indirect immunofluorescence (IIF) technique have not been fruitful [9, 66]. In one case, however, in which the patient survived PAM due to *Naegleria,* IIF test showed a titer of 1:4,096 against *N. fowleri* in serum samples obtained 7, 10, and 42 days after hospitalization [62]. An immunoblot study conducted in our laboratory indicated that the anti-*N. fowleri* antibodies are primarily IgM and that the antibodies persist for at least 4 yr (G. S. Visvesvara et al., unpubl. data).

Antibodies to *Naegleria* spp. [11, 17, 22, 30, 42, 45, 69] in apparently healthy humans have also been documented. According to one report [42], serum samples collected randomly from humans in North Carolina, Pennsylvania, and Virginia contained specific antibody for the surface membranes of particular *Naegleria* spp. The investigators in this study concluded that apparently most humans are exposed to more than one species of *Naegleria,* and persons in certain geographic areas are exposed to these antigens more extensively than those from other geographic areas. Since these amebas are so very widespread, it is not surprising that most humans, especially those who participate in water-related activities, are exposed to them. However, the significance of these antibodies in relation to their protective effects, if any, is not clear at this time.

Granulomatous amebic encephalitis due to *Acanthamoeba* or some other free-living ameba. Unlike *N. fowleri, Acanthamoeba* spp., on the other hand, cause chronic, subacute encephalitis often with granuloma formation, termed granulomatous amebic encephalitis (GAE), which also leads to death anywhere from eight days to several months after disease onset [45]. Granu-

Table 2. Granulomatous amebic encephalitis cases—United States.

No.	State	Sex	Age	Year	Agent	Skin ulcer	AIDS	Reference
1	AZ	F	6	1956	*A. culbertsoni*	+ (scalp)	–	39, 44
2	NY	M	27	1970	*A. culbertsoni*	+ (chest)	–	44
3	NY	M	57	1970	*A. culbertsoni*	–	–	38
4	PA	M	58	1971	*Acanthamoeba* sp.	–	–	44
5	TX	M	7	1972	*A. castellanii*	–	–	44
6	VA	F	47	1974	Leptomyxid[a]	–	–	24
7	LA	M	56	1975	Leptomyxid[a]	–	–	34
8	LA	F	32	1977	*A. castellanii*	–	–	44
9	CA	F	24	1977	*A. astronyxis*	+ (arm)	–	32
10	NY	F	11	1978	*A. culbertsoni*	–	–	[b]
11	SC	M	0.3	1978	Leptomyxid[a]	–	–	15
12	UT	F	57	1978	*Acanthamoeba* sp.	? (sub cut. mass)	–	31
13	TX	F	2	1978	*Acanthamoeba* sp.	–	–	[b]
14	PA	M	2.5	1979	Leptomyxid[a]	–	–	43, 76
15	PA	M	38	1980	*A. castellanii*	–	–	43
16	GA	F	40	1981	*Acanthamoeba* sp.	–	–	[b]
17	CA	F	0.8	1982	Leptomyxid	–	–	[b]
18	CA	M	72	1983	Leptomyxid	–	–	[b]
19	FL	M	61	1983	Leptomyxid	? (healed scar on knee & leg)	–	[b]
20	CA	M	34	1984	*A. culbertsoni*	+ (abdomen)	+	77
21	CA	M	34	1985	*A. castellanii*	–	+	49, 56
22	CA	M	35	1986	*A. castellanii*	+ (arm, feet)	+	[b]
23	TX	F	11	1986	Leptomyxid[a]	–	–	46
24	GA	M	34	1988	*Acanthamoeba* sp.	?	+	[b]
25	SC	M	38	1988	*Acanthamoeba* sp.	?	+	[b]
26	NY	M	36	1988	Leptomyxid	+ (nose)	+	[b]
27	MN	M	60	1988	*Acanthamoeba* sp.	?	–	[b]
28	IL	M	38	1989	*Acanthamoeba* sp.	+ (foot)	+	[b]
29	OH	M	?	1989	*Acanthamoeba* sp.	+	+	[b]
30	NY	M	38	1989	*Acanthamoeba* sp.	+	+	[b]

[a] Previously identified as *Acanthamoeba* sp.
[b] Reported to CDC.

lomatous amebic encephalitis occurs predominantly in patients who are immunosuppressed, those with diabetes or alcoholism or those receiving radiation therapy. As many as 56 cases of GAE have been reported from all over the world; 30 have occurred in the United States. Only 3 of these patients, 1 from Nigeria [14], 1 from Barbados [40] and the 3rd from India [41], have survived this disease. *Acanthamoeba* spp. have been isolated in culture in only a few cases of GAE [14, 29, 33, 40, 41, 44, 72, 73, 77]. Indirect immunofluorescence tests using anti-*Acanthamoeba* serum also have identified *Acanthamoeba* as the causative agent in only few cases [45, 72, 73]. However many cases of GAE have been identified or have been assumed to be due to *Acanthamoeba, Vahlkampfia,* or some unknown ameba in the absence of either culture isolation or serologic evidence [10, 24, 34, 46, 57]. Recently, we isolated in our laboratory a leptomyxid ameba from the brain of a baboon that died of amebic encephalitis at the San Diego Zoo Wild Animal Park. We have made antiserum against this ameba in rabbits, and by using the IIF test we have shown that as many as 14 cases of GAE, originating from different parts of the world and thought to be caused by *Acanthamoeba, Vahlkampfia,* or an unknown free-living ameba, were indeed caused by this leptomyxid ameba (G. S. Visvesvara et al., unpubl. observ.).

Granulomatous amebic encephalitis in the United States. The 1st case of GAE was reported in 1956 in a 6-year-old girl in Arizona with a skin ulcer in the scalp. However the 1960 report of this case described the disease as due to *Endolimax williamsi* (*Iodamoeba butschlii*) [39]. Since then, 29 more cases have occurred: 6 in California, 5 in New York, 3 each from Texas and Pennsylvania, 2 each in Georgia, Louisiana, and South Carolina, and 1 each from Virginia, Utah, Florida, Illinois, Ohio, and Minnesota (Fig. 2). Of these 30 cases, 10 have occurred in females and 20 in males. The average age of the females was 23.27 yr, with a range of 8.5 mo to 59 yr. The average age of the males was 33.2 yr, with a range of 4 mo to 72 yr. Almost one-third (9/30) of these patients exhibited ulcers or hard erythematous nodules in the skin (Table 2). Of these 30 cases, 9 have occurred in patients with acquired immunodeficiency syndrome (AIDS). According to the IIF data (G. S. Visvesvara et al., unpubl. data) seven of these 30 cases have been due to the leptomyxid ameba. Possibly two other cases, the case reported by Duma et al. [24] and that reported by Hoffman et al. [34] may also be due to the leptomyxid ameba.

GAE in patients with AIDS in the United States. The 1st case of GAE in a patient with AIDS occurred in 1984 in a 34-year-old man in California who had a history of chronic sinusitis, persistent diarrhea due to *Giardia lamblia* and *Cryptosporidium* sp., and chronic appendicitis [77]. He underwent an appendectomy and later developed, above the appendectomy scar, a 1-cm diameter hard nodule and also several painful, firm, widely distributed skin lesions. Amebic trophozoites and cysts were demonstrated in the skin lesions as well as in the brain. *Acanthamoeba culbertsoni* was isolated from the brain tissue [77]. Interestingly, a few months earlier, *Acanthamoeba* infection was recorded in a 29-year-old Haitian man in Florida [29], who also had AIDS. The patient exhibited thickened nasal and paranasal sinus mucosa. *Acanthamoeba castellanii* was demonstrated in a turbinate biopsy specimen which showed extensive coagulative necrosis, acute and chronic inflammatory infiltrate, and granulomatous reaction. Amebas were isolated from

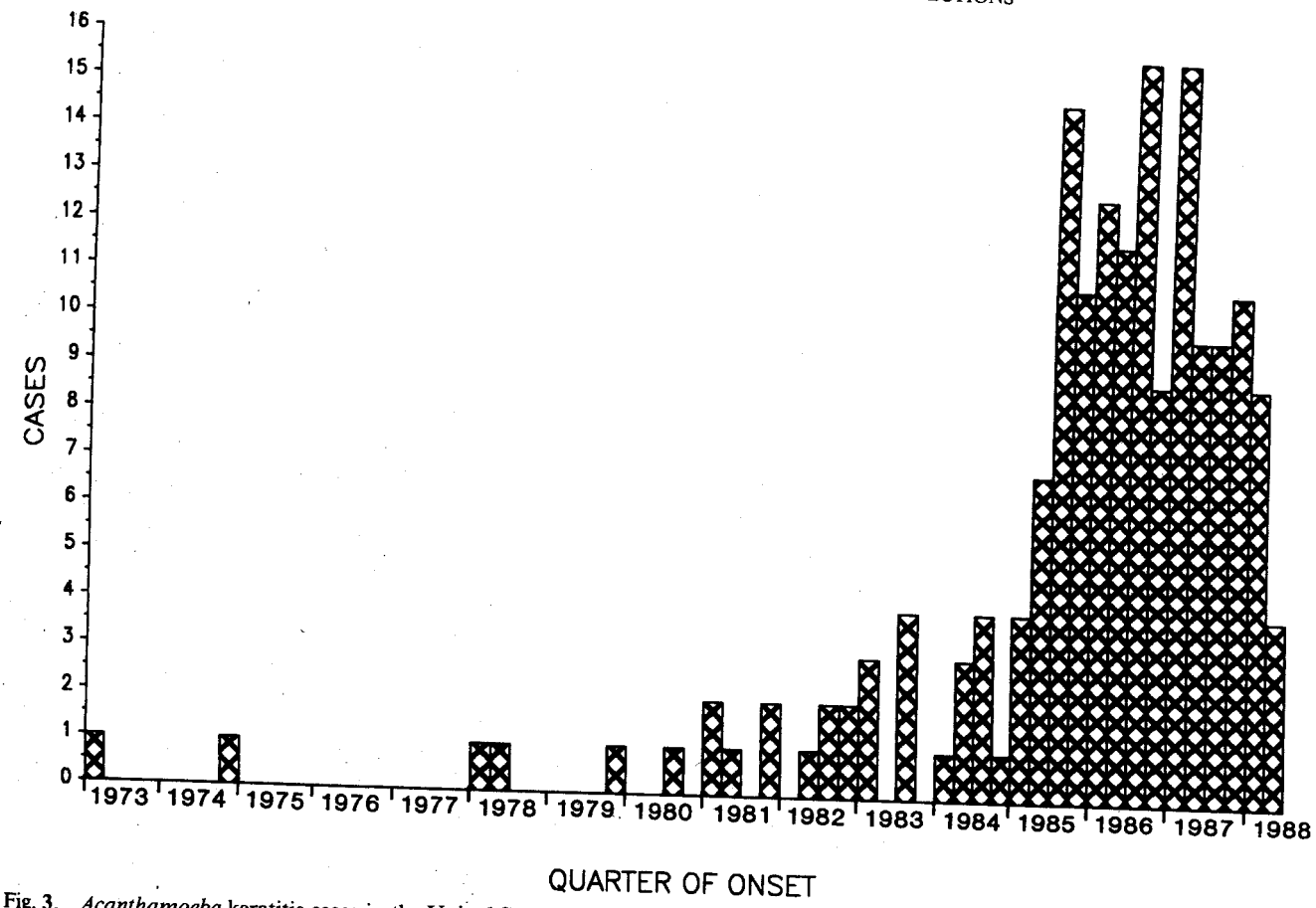

Fig. 3. *Acanthamoeba* keratitis cases in the United States by date of onset of symptoms, 1973 through June 1988. Reprinted with permission from *Amer. J. Ophthal.* [64].

the paranasal sinuses and also were demonstrated in tissue sections of the paranasal sinuses, a nodule in the calf, and an intradermal abscess in the left leg [29]. The patient also had *Pneumocystis carinii* pneumonia, *Mycobacterium avium-intracellulare* in the liver and retroperitoneal lymph nodes, cytomegalovirus infection of the adrenal glands, and Kaposi's sarcoma in the spleen. The central nervous system and the lungs, however, were not involved. Since then 8 more cases of GAE have occurred in AIDS patients, 2 each from California and New York, and 1 each from Georgia, South Carolina, Ohio, and Illinois (Table 2). All these cases have occurred in males, the average age being 35.16 yr, with a range of 34 to 38 yr.

***Acanthamoeba* keratitis in the United States.** In addition to causing GAE, *Acanthamoeba* spp. also cause a painful, vision-threatening disease of the human cornea, *Acanthamoeba* keratitis. *Acanthamoeba* keratitis has been reported from Europe, Australia, India, Taiwan, Japan, Africa, Israel, South America, and North America. *Acanthamoeba* keratitis is not a reportable disease in the United States; hence its true incidence is not known. However, published reports suggest that, although relatively rare, *Acanthamoeba* keratitis is being diagnosed with increasing frequency [8, 64, 65]. Through July 1988, 208 cases of *Acanthamoeba* keratitis in the United States had been reported to CDC [65]. The 1st case was diagnosed in 1973 in a South Texas rancher with a history of eye trauma and exposure to contaminated water [36]. Between 1973 and 1981, only five additional cases were diagnosed. The number of cases increased gradually beginning in 1981, with a dramatic increase in 1985 (Fig. 3).

Acanthamoeba keratitis has a similar distribution among men and women. Of the cases reported to CDC, 49% were in men and 51% were in women. The median age at the onset of keratitis was 29 yr, but ranged from 13 to 82 yr. Cases of *Acanthamoeba* keratitis have been reported from 34 states and the District of Columbia (Fig. 4). Most cases (41%) were reported from California, Texas, Florida, and Pennsylvania. The large number of cases from these areas is difficult to explain. Careful examination of cases from these states suggests no temporal clustering indicative of a common source of infection (such as through a contaminated lot of contact lens solution). The geographic distribution of *Acanthamoeba,* although thought to be ubiquitous, is not known. Soil and water sources from these areas may be more highly contaminated with *Acanthamoeba,* allowing more frequent exposure among persons living there. On the other hand, each of the high incidence areas has an active eye research center. Perhaps ophthalmologists from these states were more familiar with the diagnosis at an earlier time and diagnosed the disease sooner.

Historically, *Acanthamoeba* keratitis has been associated with penetrating corneal trauma and exposure to contaminated water. More recently, an association with contact lens wear has become apparent, suggesting that mechanical or hypoxic trauma may result from contact lens wear that may then allow invasion and destruction of the corneal stroma by the ameba. However, a few patients have had neither a history of trauma nor of contact lens wear. Of the 208 patients reported to CDC through July 1988, 97% had at least one risk factor for *Acanthamoeba* keratitis: 17% had a history of corneal trauma, 25% had a history

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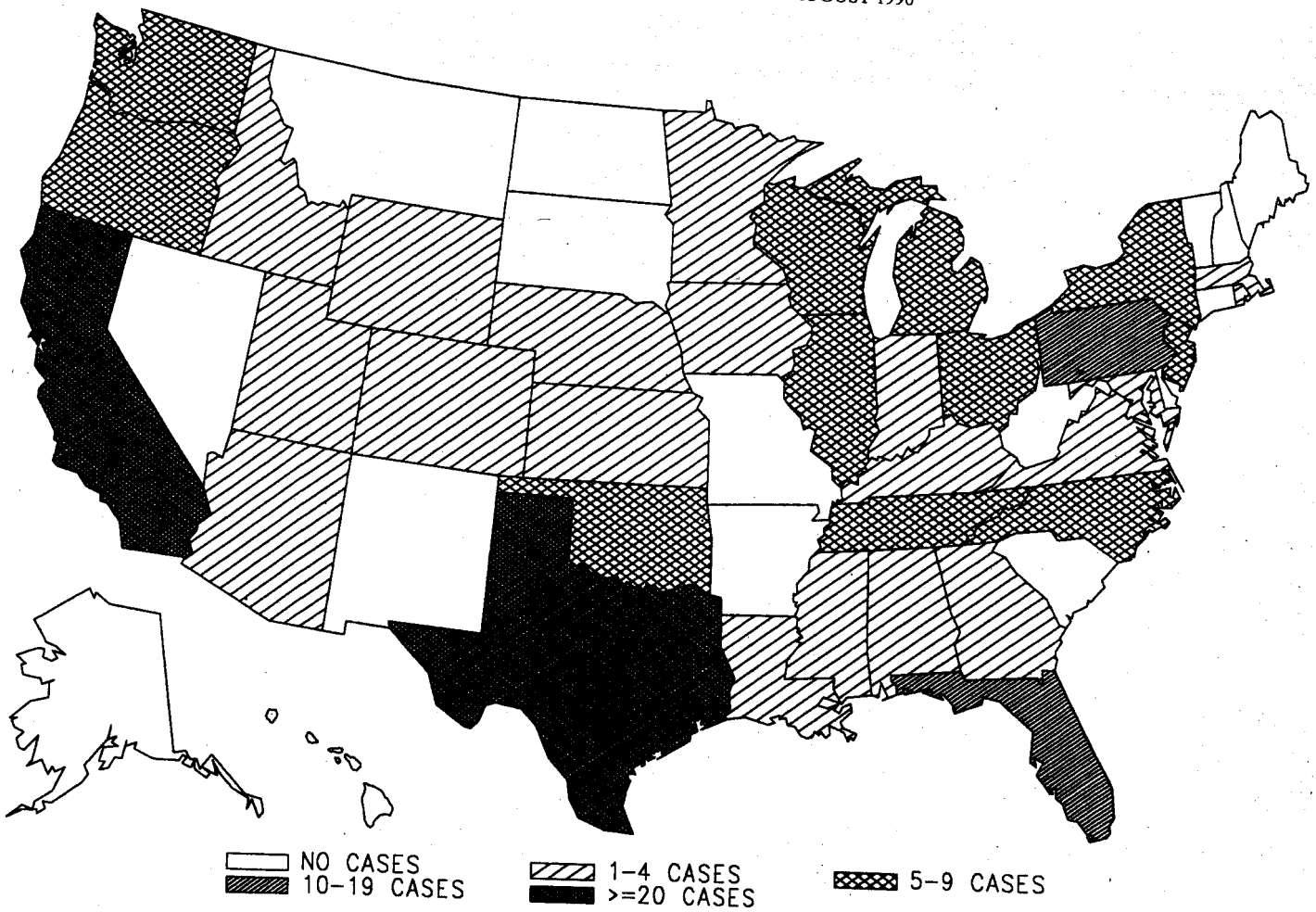

Fig. 4. *Acanthamoeba* keratitis cases in the United States—by state of residence, 1973 through June 1988. Reprinted with permission from *Amer. J. Ophthal.* [64].

of exposure to contaminated water, and 85% wore contact lenses. Elderly patients were significantly less likely to have worn contact lenses and were more likely to have had a history of corneal trauma than were younger patients. Men were more likely to have had a history of corneal trauma and less likely to have had a history of contact lens wear than were women.

The majority of cases of *Acanthamoeba* keratitis among contact lens wearers occurred in persons who wore soft contact lenses, either daily-wear or extended-wear lenses. *Acanthamoeba* keratitis in soft contact lens wearers has been associated with use of home-made saline, wearing lenses while swimming. and disinfecting lenses less frequently than recommended by lens manufacturers [65]. In most instances, these risk factors suggest a deviation from contact lens wear and care procedures recommended by the lens manufacturer and health care professionals. Commercially distributed distilled water is not sterile; therefore, home-made saline solutions reconstituted with distilled water would be expected to be contaminated with the same organisms that were present in the distilled water. As a result, home-made saline should only be used before thermal disinfection of lenses and should not be used directly in the eye. The association of *Acanthamoeba* keratitis and wearing contact lenses while swimming may have several interpretations. Most lens manufacturers recommend that lenses not be worn while swimming because of the potential for losing them. *Acanthamoeba* spp. are ubiquitous in nature, and exposure to water may provide an avenue for contamination of the eyes and the lenses with the ameba. Exposure to water may also result in increased irritation of the eye and perhaps in corneal microtrauma that may enhance the establishment of an infection. The fact that an individual wears lenses while swimming, however, may simply indicate that the wearer is less meticulous in the care of his or her lenses. Such a person may disregard other wear and care recommendations, and as a result be at an increased risk for *Acanthamoeba* during those activities.

Not much information is available on the antibody response to acanthamoebae in humans. Complement fixation (CF) antibody to *Acanthamoeba* has been demonstrated in serum samples from patients with upper respiratory tract distress and those with optic neuritis and macular disease [30, 45, 61]. Presence of antibodies to *Acanthamoeba* in patients with upper respiratory tract distress indicates that many inapparent infections due to *Acanthamoeba* may exist in nature. A study by Eldridge & Tobin [26] showed that 20% of 128 hospitalized patients had CF antibody to *Acanthamoeba*, and young children had the highest prevalence. Kenney [38] detected antibodies to *A. culbertsoni* (strain Lilly A-1) in two out of 1,000 serum samples collected from randomly selected patients. He demonstrated increasing CF antibody titers to *Acanthamoeba* in three successive samples of serum from each of two patients. The 1st sample of one of the patients, a 57-year-old man with an old brain infarct, had a 1:8 titer against *A. culbertsoni*. A 2nd serum

Table 3. Free-living ameba infection in animals.

No. Host	Agent	Tissue involved	Reference
Mammals			
1. Gorilla	Leptomyxid[a]	Brain, lungs	1
2. Baboon (*Papio sphinx*)	Leptomyxid	Brain, lungs	[b]
3. Buffalo	*Acanthamoeba*	Lungs, bronchioles	25
4. Bull (Holstein)	*Acanthamoeba*	Lungs (brain was not examined)	47
5. Bull	*A. polyphaga*	Prepucial cavity	37
6. Bull	*Acanthamoeba* sp.	Prepucial cavity	37
7. Bull	*Vahlkampfia* sp.	Prepucial cavity	37
8. Cow	*A. polyphaga*	Vagina	37
9. Sheep (Nelson big horn)	*Acanthamoeba*	Nasal mucosa and brain	16
10. Sheep (Suffolk ovine)	Leptomyxid	Brain, lungs	[b]
11. Sow	*V. inornata*	Nasal cavity	37
12. Swine	*V. avara*	Nasal cavity	37
13. Dog	*Hartmannella vermiformis*	Bronchi	37
14. Dog (greyhound)	*Acanthamoeba*	Lungs	30
15. Dog (greyhound)	*Acanthamoeba*	Lungs	30
16. Dog (Akita)	*A. castellanii*	Brain, lungs, kidney	53
17. Dog (greyhound)	*A. culbertsoni*	Brain, lungs	[b]
18. Dog (German shepherd)	*Acanthamoeba*	Heart, lung, liver, pancreas	3
19. Rabbit	*A. polyphaga*	Liver	37
Birds			
20. Domestic pigeon	*A. polyphaga*	Intestine	37
21. Turkey	*H. vermiformis*	Trachea	37
22. Turkey	*H. vermiformis*	Trachea	37
23. Turkey	*V. enterica*	Intestine	37
24. Turkey	*A. polyphaga*	Intestine	37
25. Turkey	*H. vermiformis*	Intestine	37
26. Turkey	*A. polyphaga*	Intestine	37
Fish			
27. Fish (Goldfish)	*Acanthamoeba* sp.	Kidney, liver, meninges, swim bladder	74
28. FISH (11 spp. belonging to 8 genera)	*Acanthamoeba* sp.	Gills, urinary bladder, spleen, gall bladder, blood	68
	Vahlkampfia sp.		
29. Rainbow trout	*Naegleria* sp.		
	Vexellifera bacillipedes		60
Invertebrates			
30. Snail (*Bulinus* spp. & *Biomphalaria* spp.)	*H. biparia* & *H. quadriparia*	Mantle collar, foot & intestinal wall, tentacles, mantle collar, foot	55
31. Grey crab (*Callinectes sapidus*)	*Paramoeba perniciosa*	Blood and skeletal & cardiac muscle tissue, hepatopancreas	63
32. Rock crab (*Cancer irroratus*)	*P. perniciosa*		59
33. Lobster (*Homarus americanus*)	*P. perniciosa*		59
34. Oyster (*Crassostrea commercialis*)	*H. tahitiensis*		13
35. Sporocysts of *Schistosoma mansoni*	*Nuclearia* sp.	Tegumental membrane	52

[a] Previously identifed as *Acanthamoeba*.
[b] Reported to CDC.

sample taken 1 mo later showed an increase in titer to 1:16. A 3rd sample, taken 1 mo after the 2nd, showed a further increase in the titer to 1:64. The patient later died of cerebral hemorrhage, and amebas were demonstrated in the brain section. Three successive serum samples taken 1 mo apart from a 39-year-old man hospitalized with acute gastritis also showed an increase from 2 to 16 in the CF titers to *A. culbertsoni* antigens. Amebas were seen in the stool samples of this patient, but were identified as *Iodamoeba butschlii*. The patient was treated with dehydroemetine and chloroquine, and the CF titer decreased to 1:2 in a serum sample obtained 2 mo later.

Antibodies to *Acanthamoeba* have also been demonstrated in the serum of *Acanthamoeba* keratitis patients, using the Ouchterlony precipitin as well as the more sensitive IIF tests [4, 36, 58, 70, 71]. Recently, a four-fold increase in the ameba-immobilization antibody titer to *A. rhysodes*, over a 16-mo period, was demonstrated in the serum of a Nigerian patient, who made a partial recovery from an *A. rhysodes*-induced CNS disease [14]. *Acanthamoeba rhysodes* was repeatedly recovered from the CSF of this patient. Antibodies to *Acanthamoeba* have also been demonstrated in apparently healthy humans [17, 30, 45]. Recently, Cerva [11] surveyed 1,054 people in Czechoslovakia and found low levels of antibodies to *A. culbertsoni* and *N. fowleri*. However, he found significant levels of antibodies to *A. culbertsoni* in 52% of patients with hepatitis A infection as well as in convalescents.

Acanthamoebae have also been isolated from sites other than the CNS, cornea, sinuses and the skin. For example, a strain of *A. castellanii* was isolated from pus and necrotic tissue of a patient with submandibular cellulitis [5, 72]. The patient, a 32-

year-old woman with a history of possible diabetes, developed ameloblastoma on the right side of the mandible, which was resected, and an autograft from the right iliac crest was used to fill the defect. The graft became infected with gram-negative cocci and gram-positive and gram-negative bacilli and *Acanthamoeba*. The patient recovered from the infection after the surgical excision of the infected portion of the graft, intravenous penicillin therapy, and betadine mouth wash.

Acanthamoeba spp. have also been isolated from bronchial secretions, stool samples of patients with diarrhea, and from ear discharge [22, 30, 45]. Griffin isolated 5 *Acanthamoeba* strains from 8 stool samples, and Jadin isolated 7 strains of *Acanthamoeba* from diarrheic stool samples of patients, mostly children [30]. Two of these seven strains of *Acanthamoeba* were pathogenic and killed mice. It is quite likely that under certain conditions, some strains of *Acanthamoeba* may cause transitory infection and thereby stimulate host defense mechanisms which control the infection and eliminate the causative organism. This also indicates that acanthamoebae are quite susceptible to human defense mechanisms in normal individuals. These amebas may, however, cause severe problems in immunologically compromised or otherwise debilitated individuals. Griffin found free-living amebas, probably *Acanthamoeba,* in three separate Pap smears, and Dyner detected free-living amoebas in urine samples of several immunosuppressed patients [30].

Free-living ameba infections in animals. Infections due to *Acanthamoeba* have been reported in a variety of animals, e.g. gorillas, buffalo, bull, sheep, dog, fish (Table 3). Based on the IIF data, the infection in the gorilla as well as in the baboon and one of the sheep (Suffolk ovine) is indeed due to the leptomyxid ameba (G. S. Visvesvara et al., unpubl. observ.). Besides *Acanthamoeba* and *Naegleria,* other free-living amebas belonging to such genera as *Hartmannella, Paramoeba, Vexellifera, Nuclearia* have been implicated in disease in a variety of invertebrates (Table 3).

These observations, together with the fact that *Acanthamoeba* spp., *Naegleria fowleri,* and *Hartmannella* sp. can harbor pathogenic microorganisms such as *Legionella* [22, 27, 51] and or mycabacteria [35] and that *Acanthamoeba* sp. and *Hartmannella* sp. have been used to isolate *Legionella* sp. when it may not be possible to isolate legionallae directly from the environment indicate the public health importance of these organisms.

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