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1 **Is *Naegleria fowleri* an Emerging Parasite?**

2

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12

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14

15 **Abstract**

16 *Naegleria fowleri* causes an uncommon but deadly disease called primary amoebic
17 meningoencephalitis (PAM). There has been an increase of reported PAM cases, particularly
18 since 2000. Although water is the dominant route of transmission of PAM, infection through
19 soil/dust is a possible alternative route. We have observed differences in epidemiology between
20 the southern US states and the Indian subcontinent (ISC). The patient age range is greater in
21 ISC than the US, and there are more infections in ISC which are not water-associated. We show
22 that PAM is under reported and argue that climate change will increase the incidence of PAM
23 and the geographic range of *N. fowleri* will spread poleward.

24

25

26 **Pathogenic amoebae**

27 The obligate parasitic amoeba *Entamoeba histolytica* causes huge human mortality, but the
28 **free-living amoebae (FLA, see Glossary)** in the same supergroup amoebozoa, *Acanthamoeba*,
29 *Balamuthia* and *Sappinia*, are known to cause encephalitis in humans [1], while the excavate
30 amoeba *Naegleria fowleri* (Box 1) causes meningoencephalitis [2]. Members of the
31 **amoeboflagellated** genus *Naegleria* are common throughout the world, particularly in soils
32 and freshwater. The genus was named to honour the German protozoologist Kurt Nägler [3]
33 and currently contains about 50 recognised species. Only one of these, *N. fowleri*, named for
34 Malcolm Fowler [4], is a confirmed human pathogen. Fowler and Carter first described the
35 disease [2], which was later named primary amoebic meningoencephalitis (PAM) by Cecil Butt
36 [5] (Box 2). The term “primary” is used as the amoebae are the root cause of the disease, and
37 “meningoencephalitis” is an encephalitis that also involves inflammation of the meninges.

38

39 **Is *Naegleria fowleri* an emerging pathogen?**

40 An emerging infectious disease is defined as a disease whose incidence has increased in the
41 past 20 years and which seems likely to increase further (CDC Atlanta). *N. fowleri* has been
42 described as an emerging parasite by some [6], and an emerging medical threat by others [7].
43 We have searched the literature to find as many PAM cases as possible, looking for trends in
44 reported infections. A previous survey of PAM laboratory-confirmed cases in the US between
45 1962 and 2008 showed variation from year to year but no overall increase in incidence was
46 claimed [8], however looking at the worldwide data over a longer period (Figure 1A) we
47 suggest that there has been an increase in reported cases, especially since 2000. However, we
48 do not know if the incidence of the disease is actually increasing or if the increasing numbers
49 of publications merely reflects its increasing recognition and notoriety, especially since the
50 evocative name “brain-eating amoeba” has been adopted by the media and by some biologists
51 [9]. Whatever the reason, we argue that PAM qualifies for labelling as an emerging infectious
52 disease, since it seems inevitable that its actual incidence will increase due to climate change.

53

54 **Patterns of primary amoebic meningoencephalitis**

55 Much of what is known about PAM has been gleaned from cases in the USA, but differences
56 between the world’s regions are to be expected due to local geography and human habits. We
57 have found a difference in the patterns of PAM victims between the USA and the Indian
58 subcontinent (ISC), USA victims tending to be younger than those from ISC (Figure 1B) with

59 the exception of victims under 1 years old, for which the recorded rate was 7 times greater in
60 ISC than in the USA. The age spread within the ISC is also greater than the USA population
61 (Figure 1B). The world-wide gender bias shows that there are more male than female cases
62 (Figure 1C) but we have found that this bias is greater in the ISC group, with 14 times more
63 males than females, compared to 3 times more in the USA group. The typical US victim is a
64 young male who becomes exposed to *N. fowleri* in recreational situations. Boys are more likely
65 to play in water and to be more boisterous as they do so, immersing themselves and stirring up
66 lake sediments containing *N. fowleri* **trophozoites** and **cysts**, which explains the gender bias.
67 In the ISC, contact with *N. fowleri* seems to be through play but also through ablution rituals,
68 washing and a lack of chlorination, meaning that the general population is at risk from PAM,
69 not just those who play in natural bodies of water, for which chlorination is not feasible.

70

71 **“Wet” vs “dry” infection**

72 We calculate that where possible sources of *N. fowleri* have been reported, 93% of PAM cases
73 are described as infections that have resulted from exposure to water (314 cases out of 336).
74 Water is clearly the most frequent route of infection in most cases, but it has been argued that
75 people can also be infected through cyst-laden dust entering the nasal passages and then
76 excysting, leading to infection [10, 11]. These **“dry infections”** are particularly worrying since
77 there is little that people living in such areas can do to avoid cyst inhalation. Infections with
78 cysts (dry infections) account for 6.5% of PAM cases where possible sources are reported.
79 Cysts may also enter the **nares** through the **nasolacrimal ducts** when contaminated dust alights
80 on the eye. Although the dry infection route has been doubted by some [12] who point out that
81 *N. fowleri* cysts last about 5 minutes when desiccated [13], we have found that amoeba cysts
82 survive for years when dried in fine clay [14], and so it is likely that fine clay improves cyst
83 survival in air. We have found too that the dry infections tend to be in warm/hot regions (Figure
84 2). The incidence of nasal carriage, which is discussed later, is fortunately higher than the
85 development of PAM, suggesting that there may be a threshold effect in which large numbers
86 of activated amoebae are required to penetrate the epithelium and invade the brain, so that a
87 low cyst carriage does not represent a high risk. Physical trauma to the nasal epithelium may
88 also be important in PAM development through dry infection. Another marked difference
89 between PAM cases from the USA and the ISC is that the latter are more likely to have been
90 suspected dry infections (0% in USA vs 8% in ISC).

91

92 **Where is *N. fowleri* and where is PAM?**

93 *N. fowleri* is known to be distributed throughout all continents except Antarctica and is
94 especially common in the warmer equatorial countries [15]. In temperate countries such as
95 those in Northern Europe, *N. fowleri* is typically found in waters that are warmed either
96 artificially (e.g. cooling towers from power stations) or naturally (e.g. **geothermal** areas).
97 Cases of PAM are typically restricted to warm countries and their distribution correlates most
98 strongly with areas where average annual temperatures lie between 15 – 18°C (Figure 2). Many
99 have reported that PAM is highly seasonal away from equatorial regions [16]. The distribution
100 of PAM largely reflects that of human populations within the warmer regions, although the
101 ISC and Southeast Asia are notable exceptions, as here higher PAM incidence is found together
102 with high temperatures. Many of the European recorded cases are not recent and have occurred
103 in natural warm springs (e.g. City of Bath, England), or artificially heated areas such as power
104 stations and baths (Belgium, Czech Republic), however more recent cases in Minnesota (white
105 arrow, Figure 2) occurred far from other cases and in a natural lake, leading to concerns that
106 *N. fowleri* may now be occurring further north due to climate change [17]. Another northerly
107 case from N.E Pennsylvania [18] has been questioned [19].

108 It is important to understand what determines the distribution and abundance of *N. fowleri* in
109 the environment and which niches these amoebae occupy within the ecosystems. This is known
110 to be influenced by factors such as temperature, salinity, the availability of suitable prey
111 organisms and the presence of amoeba pathogens, including some bacteria and viruses. A
112 further, less-explored consideration is that of competition with other amoebae, especially other
113 *Naegleria* species [20].

114

115 *Temperature*

116 *N. fowleri* is associated with elevated temperatures (Figures 2 & 3). It is found in regions where
117 water/soils are warm, such as geothermal sources, or where human activities result in water
118 bodies that are warm over extended periods. *N. fowleri* grows at temperatures of 30° - 46°C
119 [21], but in **biofilms** it is reported that the amoebae need temperatures of 42°C to support
120 growth and that 32°C was insufficient [22]. Amoebae remained viable for 24hrs at 49°C but
121 were nonviable by 48 h [23]. Trophozoites and *N. fowleri* cysts can also survive for a few
122 minutes to hours at 50-65°C, with cysts being more resistant to the effects of high temperatures
123 than the trophozoites are [13]. *N. fowleri* trophozoites degenerate within hours below 10°C
124 [13], while the cysts can survive at 4°C for six months [24] meaning that they can potentially
125 overwinter in lakes/rivers and grow the following summer.

126

127 *Salinity, osmotic tolerance*

128 *N. fowleri* must be capable of tolerating human physiological ion concentrations to survive
129 passage through the **cribiform plate** into the brain. The tonicity of human tissues is equivalent
130 to 0.9% NaCl, and *N. fowleri* trophozoites are reported to remain viable up to 1.4% [23] or
131 even 2% [25]. It therefore seems that *N. fowleri* is quite capable of adapting to survive the
132 salinity of human tissue. Fresh-water amoebae adapt to higher salinity over time [26] and
133 because the period of exposure and expression of disease symptoms is so short, this adaptation
134 probably takes place with the individual amoebae causing the infection rather than their
135 progeny. In mice, *N. fowleri* trophozoites are found within the olfactory bulb as early as 24hr
136 after nasal inoculation [27].

137

138 **The distribution of *N. fowleri* in water bodies**

139 The availability of suitable prey is likely to be an important determinant of *N. fowleri*
140 distribution in warm lakes (Figure 4). FLA are found in large numbers in floating biofilms at
141 the meniscus with bacteria [28] and *N. fowleri* are enriched here too [29, 30]. **Cyanobacteria**
142 are extensively grazed by protists [31] especially amoebae [32]. *Naegleria* (and other FLA)
143 are associated with cyanobacteria-dominated layers in a stratified lake [33] and *N. australiensis*
144 is known to prey on a range of cyanobacteria (but not all) [34]. Cyanobacterial blooms can
145 maintain a position in the water column as they can regulate their buoyancy through gas
146 vesicles and trophozoites are able to crawl amongst these floating masses as they prey upon
147 them. While *N. fowleri* trophozoites are found associated with these bacteria in the water
148 column, free swimming *N. fowleri* **flagellates** are also found here. Amoebae prey on bacteria
149 in two-dimensional biofilms much more efficiently than they can on cells in suspension [35]
150 and the *N. fowleri* flagellate cannot feed at all, so that it is expected that much of their feeding
151 and growth takes place on the lake floor within the sediment. **Groundwater** systems occupy
152 vast volumes with huge biofilm potential, often with elevated temperatures. In Arizona *N.*
153 *fowleri* has been found in groundwater [36], and the temperature varied between 13 and 46°C
154 in the wells that draw on this water source [37, 38]. In Australia, too, the amoeba is found in
155 groundwater and where this breaks the surface, such as in **mound springs** and bore holes [39].
156 Groundwater may well be the largest natural niche for *N. fowleri* and the most important
157 reservoir for re-infection of surface waters (Figure 4).

158

159 **Climate change and the rise of *N. fowleri***

160

161 Climate models predict that almost everywhere will become warmer, and a global rise in
162 average temperatures has already been recorded. It is also predicted [40] that large areas will
163 experience increased drought conditions (USA, Central America, Brazil, North Africa, the
164 Mediterranean region, Australia, South China). Other areas (Northern Europe, Central East
165 Africa, the Indian subcontinent, Northern Eurasia) will see increased precipitation which will
166 cause increased erosion and eutrophication of freshwater courses. Perhaps counterintuitively
167 both drought and increased precipitation may favour *N. fowleri* and the spread of PAM, through
168 different mechanisms. Drought may make the use of rooftop rainwater systems more attractive
169 in some areas and in others the use of artesian wells drawing on infected ground-water is
170 expected to increase the risk of *N. fowleri* exposure and PAM. Concerns around the
171 microbiological safety of roof-harvested rainwater have already been raised [41], and *N.*
172 *fowleri* is often detected in roof-harvested rainwater tanks in Australia and South Africa [42].
173 Although there is an abundance of evidence to suggest that global warming is real, does this
174 actually mean that the habitat favoured by *N. fowleri* is increasing together with a population
175 increase? This amoeba seems to have an advantage over related organisms when temperatures
176 are above 30°C (Figure 3). Many claims have been made in the literature of a connection
177 between global warming and PAM [7, 16, 43,]. Given the thermophilic nature of *N. fowleri*
178 this is an attractively simple idea, but what is the evidence for this connection? It has been
179 observed that in the US, the rate of PAM is distinctly seasonal [8,16] and this has also been
180 reported in Australia [12] and in Karachi [44], but here screening for PAM is routinely
181 undertaken only in the summer months, so the observed seasonality may have been exaggerated
182 in Karachi. Several cases of PAM specifically mention that the patients had sought relief from
183 the heat by immersing themselves in the water that has been blamed for their subsequent
184 infection [45]. In general, the seasonality of PAM suggests a causal link between higher
185 temperatures and greater incidence of disease, as indicated too by the geographic occurrences.

186

187

188 **Are we only seeing the tip of a PAM iceberg?**

189 There is a strong suspicion that PAM is very much more common than currently indicated,
190 especially in the developing world where conditions for *N. fowleri* tend to be favourable and
191 where PAM maybe masked by a myriad of other diseases and by economic deprivation. PAM
192 is under-reported, as many cases are misdiagnosed as viral or bacterial meningoencephalitis
193 and autopsies are not always carried out, either through lack of time or because family members
194 deny permission [46]. With approximately 3 PAM cases typically reported annually in the

195 USA, it is estimated that there are likely to be a further 13 unreported cases [47]. Although
196 PAM is considered a rare disease, it has been associated with outbreaks involving many deaths,
197 for example, 16 individuals fell victim to PAM from a single swimming pool in what is now
198 the Czech Republic [48] and an outbreak in Karachi during 2015 – 2017 has claimed at least
199 24 lives [49]. It is probable that, particularly in the developing world, many more such
200 outbreaks have occurred and have gone unreported in the biomedical literature due to a lack of
201 expertise in local medical staff [50]. A further reason why incidences of PAM may be high in
202 the hot dry regions of Africa for example is the presence *N. fowleri* in the nares (nasal passages)
203 of non-infected people.

204

205 **Assessing the risk of PAM from nasal *N. fowleri* carriage**

206 Although it is reported that the most common route of entry into the human host is through the
207 nose by contact with trophozoite- laden water (**wet infection**), it is also possible that dry cysts
208 in dust can infect (dry infection) [51]. Two cases from Northern Nigeria arose in the very dry
209 conditions of the **harmattan season** [11], while *N. fowleri* was recovered from the air during
210 the harmattan [52] and have been isolated from the nares of 2 out of 50 non-infected children
211 during the hartmattan in Zaria, Nigeria [53]. Additionally, a separate study some 400 miles
212 away, in Borno State, Nigeria, found 3 out of 50 children carrying viable *N. fowleri* in their
213 nares in [54], while none were found in the nares of 1039 people from the Brest region of
214 Brittany, France [55], none in 262 students from Flinders, southern Australia [56], none in
215 1,551 army recruits from North Bohemia [57] and none in 500 children in Alexandria [58]. It
216 seems highly probable that carriage of *N. fowleri* in the nares increases the risk of PAM, so that
217 the incidence of the disease is likely to be very much greater in warm, dry regions such as
218 Nigeria and India (Figure 2).

219

220

221 **Concluding remarks**

222 Many countries are already recording extreme temperatures and many people are seeking relief
223 from the heat by immersing themselves in water that is often very warm and polluted by
224 **coliforms**, perfect conditions for the growth of *N. fowleri*. People in some of the same areas
225 practise cleansing rituals in which water is forced through the nares by specialised **neti pot**
226 devices or other means. This is believed to be the cause of the alarming increase in the incidence
227 of PAM in cities such as Karachi [7, 59]. However, it is very likely that other cities are also

228 experiencing silent outbreaks and that Karachi has become a known hotspot for PAM solely
229 because of local expertise in PAM diagnoses. Sensitive education is needed to raise awareness
230 of PAM, in the hope that simple alterations in practice, such as the use of water which has been
231 boiled then cooled for neti pots, may prevent *N. fowleri* infections. We cannot presently state
232 with certainty that *N. fowleri* is an actual emerging pathogen since we do not know
233 unequivocally that the actual number of infections is increasing or has increased over the past
234 20 years, although this remains a distinct possibility (see Outstanding Questions). There does
235 seem to be good reason to suspect that the number of *N. fowleri* cases will increase as the
236 human population approaches its expected peak, especially in some of the regions where
237 conditions seem most suitable for *N. fowleri* growth (UN Population Division 2017) and so the
238 label of emerging pathogen is warranted on those grounds alone. We expect that the gender
239 imbalance in cases will remain. Other free-living thermophilic amoebae, such as *Balamuthia*
240 *mandrillaris* and some *Acanthamoeba* strains, also cause encephalitis and these too are
241 expected to increase as temperatures increase, making awareness of pathogenic amoebae in
242 general more important. A better understanding of the parameters that dictate the presence and
243 abundance of the pathogens is needed. It is also clear that better options for their diagnosis,
244 treatment and eradication are required.

245

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251

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410

411 **Glossary**

412 **Amoeboflagellate:** An amoeba that can transform reversibly into a flagellate. The genus
413 *Naegleria* is a well-known amoeboflagellate.

414 **Biofilm:** A biofilm is a layer of cells usually dominated by bacteria which associate on a
415 surface. An extracellular layer of proteins and polysaccharides is often produced by the
416 community which may be grazed by eukaryotes such as amoebae which prefer to feed on
417 surfaces.

418 **Cerebrospinal fluid (CSF):** The liquid percolating the brain and spinal cord.

419 **Coliform:** A group of gram-negative rod-shaped bacteria that ferment
420 lactose. Faecal *coliforms* are *coliforms* typically present in the gut and faeces of birds and
421 mammals.

422 **Cribiform plate:** A sieve-like extension of the ethmoid bone which supports the olfactory bulb
423 of the brain.

424 **Cyanobacteria:** A group of photosynthetic bacteria that produce oxygen. They were known
425 as the blue green algae although this term is now disfavoured as they are not algae.

426 **Cyst:** A resting non-dividing stage formed by many protists including *Naegleria*. The
427 *Naegleria* cyst is usually has a single nucleus and a protective cell wall.

428 **Dry infection and wet infection:** A wet infection is defined by the patient receiving *N. fowleri*
429 infection from an aqueous source introduced directly to the nares. A dry infection is defined as
430 *N. fowleri* being introduced to the nares probably as cysts within inhaled dust or sand. For each
431 PAM case, we have reviewed the literature and classified them as being either wet (usually) or
432 dry where there is sufficient information as to how the disease was acquired. In most cases the
433 source cannot be absolutely verified but we have been guided by the author's opinion as to the
434 most likely source or situation. While the majority of cases are wet, it is important not to
435 exclude the possibility that dry infections can occur under specific conditions.

436 **Flagellate:** A cell that is capable of swimming through the possession of one or more flagella.
437 The *Naegleria fowleri* flagellate stage typically has two flagella.

438 **Free-living amoebae (FLA):** Those amoebae capable of living without parasitizing metazoans
439 but some of which have that ability. The term is used principally to differentiate this group
440 from others such as Entamoebae (predominantly obligate parasites).

441 **Fulminant:** In this context a disease of rapid onset and escalation. From Latin fulmināre, to
442 strike with lightning.

443 **Geothermal:** Heat originating from the Earth's core produced by radioactive decay

444 **Groundwater:** The water that lies beneath the Earth's surface in voids of various sizes between
445 soil particles, in rock fissures and in subterranean lakes.

446 **Harmattan season:** The name given to the season between November and March in West
447 Africa when dry and dusty air blows from the Sahara Desert.

448 **Mound springs:** Primarily an Australian phenomenon in which distinctive mounds are pushed
449 up by springs gushing to the surface from groundwater.

450 **Nares:** The openings of the nose and nasal cavity

451 **Nasolacrimal ducts:** Also called the "tear duct" they carry tears from the eye to the nasal
452 cavity.

453 **Neti pot:** A device used to facilitate the entry of water into the nares in an act of personal
454 hygiene, as a religious act or both.

455 **Trophozoite:** The actively moving amoeboid stage of the organism.

456

457 **Box 1. *Naegleria fowleri*, the pathogen**

458 As is typical for the genus, *N. fowleri* exists in three distinct phases (Figure I): the resting cyst,
459 a swimming flagellate and the active multiplying amoeba stage, also called the trophozoite
460 [60]. Only the latter feeds and can invade the human host, although the flagellate is sometimes
461 observed in **cerebrospinal fluid (CSF)** [45]. In active locomotion, the trophozoite is about
462 22µm in length and 7µm wide and the locomotory rate is about 45µm /min at 37°C [4]. The
463 nucleus has a distinctive appearance with concentric nucleolus (inner) and karyosome (outer)
464 regions. The flagellate is pear-shaped and 15µm long, with two apical flagella around 12µm in
465 length. The presence of the flagellate stage has been used with other features to identify *N.*
466 *fowleri* [61], but such identification is unsafe, as many strains do not produce flagellates under
467 the conditions of this test [61, 62]. The cysts are between 7 and 15 µm in diameter and unlike
468 some other *Naegleria*, do not have visible pores in the wall (although they have been observed
469 under electron microscopy). The nucleus is often not visible in the cysts of *N. fowleri*, whereas
470 it is in other *Naegleria*. Figure I shows how the stages are related to each other and the
471 approximate times taken to transform between them under conducive conditions. *N. fowleri*
472 can be differentiated from other FLA in brain tissue. *Acanthamoeba* is only rarely found in the
473 CSF [1] and *Acanthamoeba* cysts are frequently seen in human brain tissue while *N. fowleri*
474 cysts are not typically seen [63].

475

476 **Figure I (in Box 1). The life cycle of *Naegleria fowleri*.** The trophozoite at amoeboid stage
477 is motile through its actin-based cytoskeleton and crawls around eating bacteria (or host cells
478 in its parasitic mode). This is the reproductive stage and it can differentiate into a flagellate, or
479 a cyst. The flagellate can swim through its two (usually) flagella that are microtubule-based.
480 The cyst is a resting stage which is adopted during stressful situations such as suboptimal
481 temperatures or lack of prey. The time taken to differentiate between these stages are shown.

482

483 **Box 2. Primary amoebic meningoencephalitis (PAM), the disease.**

484 PAM is usually described as being a rare disease and it is certainly rarely reported, as we have
485 been able to find only 431 cases in the literature. However, there is a tendency for PAM to
486 occur in outbreaks. 16 cases arose in one outbreak in a swimming pool [48] and 3 lethal PAM
487 cases are suspected and only one confirmed over a 14-year period, in a “tiny population”
488 centred around a Queensland cattle station [39]. These examples attest both to the under-
489 diagnosed nature of PAM and to the persistence of the parasite in areas that suit it. The
490 supposed rarity of the **fulminant** disease PAM must also be set against its depressingly high

491 mortality rate, and it is still usually diagnosed *post-mortem* [64]. There is a risk that outbreaks
492 larger than those already documented may occur if vigilance fails.

493 Symptoms and diagnosis of PAM Patients often present with headaches, sore throat, blocked
494 nose, fever, nausea, altered taste and smell perception and photophobia [59, 65]. Projectile
495 vomiting may occur [46], caused by increased intracranial pressure activating the *area*
496 *postrema* of the *medulla oblongata*. Lethargy and irritability often subside into
497 unconsciousness and coma. PAM can easily be mistaken for other meningitises, and the
498 symptoms of PAM and bacterial meningitis are very similar [66]. An early diagnosis is
499 essential because of the very rapid development of the infection. High-powered microscopes
500 are necessary to differentiate the motile amoebae in CSF from neutrophils and this should be
501 done as soon as possible after lumbar puncture, as the amoebae quickly degenerate outside the
502 body unless treated carefully [67]. Skill is required to culture the amoebae from clinical
503 samples, and this is a time-consuming activity. PCR diagnosis is effective if the correct primers
504 are immediately available, but this is usually used to confirm a lethal infection rather than to
505 guide clinical practise in treatment.

506 PAM is usually contracted through exposure to water, when the amoebae contact the nasal
507 epithelium of the human host. The incubation period after exposure is 3-8 days, after which the
508 disease develops rapidly, the patient typically dying 7-10 days after the first appearance of
509 symptoms. There is no indication that PAM is infective between humans, although lab
510 experiments suggest that mouse-to-mouse transmission of *N. fowleri* may occur [68].

511 A 95% mortality rate is often quoted for PAM [69] and we have found a rate of 94.6% based
512 on the 431 cases analysed here, however among the 21 cases in which PAM was successfully
513 treated, there are some in which the involvement of *N. fowleri* is questionable. For example, in
514 the surviving PAM case in Allahabad, Pakistan, *N. fowleri* was only identified microscopically
515 [70], this was also true in other apparently successful cases [71]. If we remove those apparently
516 successful cases where there is reason to doubt the identity of the pathogen, the mortality rate
517 rises to 96.4% for the cases analysed here.

518

519

520 **Figure Legends**

521 **Figure 1. Global PAM status.** **A.** Numbers of reported PAM cases from the literatures world-
522 wide between 1961-2018. Years are from either when cases were recorded at hospitals, or in
523 the absence of this information, the years of the publication of the report. The incidence of
524 reports shows an increasing trend after around 2000 in world-wide totals whereas the USA
525 report shows a less marked increase. **B.** USA age profile (blue) vs Indian subcontinent (ISC)
526 age profile (orange) of PAM cases. There are more old victims from ISC than from the USA.
527 **C.** World-wide gender and age bias. There are more male cases (blue) than female case
528 (orange) but the overall age distribution is similar.

529

530 **Figure 2. Worldwide distribution of reported PAM cases.** Cases are clearly limited to the
531 warmer parts of the world, but the extremely warm areas tend to show less cases in line with
532 previous reports [15]. A recent northerly case [17] (white arrow) may suggest an expansion to
533 the range in the US. However, it is noticeable that the dry infections (blue dots) appear to be
534 associated with warmer areas than the wet infections (black dots). The atlas shows the annual
535 average surface temperature (°C). Used by permission of The Center for Sustainability and the
536 Global Environment, Nelson Institute for Environmental Studies, University of Wisconsin-
537 Madison.

538

539 **Figure 3 The temperature range of *Naegleria sp.*** Note that the lower range for growth tends
540 to be less well characterised than the upper temperature.

541

542 **Figure 4. A summary of the habitats occupied by *N. fowleri*.** The amoebae flourish in warm
543 ground water bodies [39]. *N. fowleri* exists in the warm groundwater in all three forms. The
544 groundwater is taken to the surface by boreholes or by natural areas such as Australian mound
545 ponds to infect warm rivers and lakes. Amoebae on the surface transform into cysts which may
546 then be infective, especially when airborne. Amoebae may exist in the surface biofilm, in
547 association with mid-water buoyant cyanobacteria and on and in the sediment. Flagellates are
548 found throughout the water column.

549

550