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6. Pathogenic *Naegleria* amoebae in the waters of Bath: a fatality and its consequences

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In July 1978 a child was admitted to the Royal United Hospital, Bath, apparently suffering from bacterial meningitis. Standard antibiotic therapy was instituted but produced no improvement. During a routine cell count on the cerebro-spinal fluid, amoebae were observed by an alert MLSO, Alan Yates, moving actively in the counting-chamber.

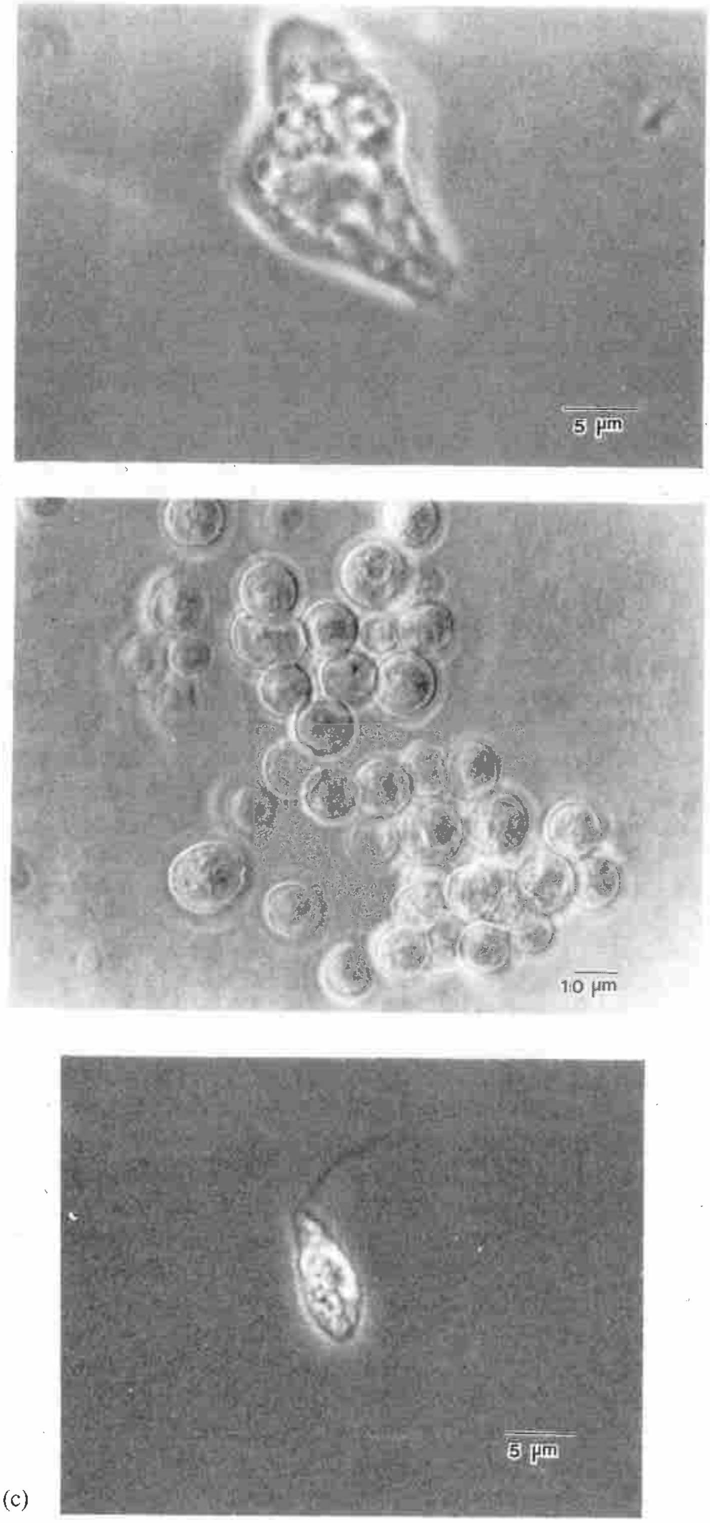
It was realized that the patient had participated a few days earlier in a swimming gala at the Beau Street baths. The organism was identified presumptively as *Naegleria fowleri*, a small free-living amoeba generally acquired from thermal water sources. Appropriate therapy with amphotericin was begun, but although amoebae were eliminated from the CSF the patient died on the ninth day of illness from damage already inflicted on the brain (Cain *et al.* 1981). This has been the unfortunate outcome of nearly all the cases of *Naegleria* infection reported throughout the world.

A review of the world literature on pathogenic small free-living amoebae has been published (Warhurst 1985) and can be consulted for references to points made in what follows. The pathogenic potential of free-living amoebae was discovered by Clyde Culbertson in 1958, when working on Salk poliomyelitis vaccine. Culbertson and his colleagues found that a free-living soil amoeba (*Acanthamoeba*) contaminating kidney tissue-culture cells produced fatal meningoencephalitis in mice and monkeys when instilled intranasally. In the 1960s reports of acute, fatal human brain infections due to free-living amoebae came from Australia, Czechoslovakia and the United States. The organism responsible was eventually identified as a species of *Naegleria*. *Acanthamoeba* was also recognized as the cause of a more chronic, fatal brain disease in man, mainly in the immuno-suppressed, as in Hodgkin's Disease and, latterly, in AIDS. The same amoeba also causes intractable corneal ulcers in immuno-competent individuals.

Both *Naegleria* and *Acanthamoeba* are found living normally in soil and water in the absence of a human or animal host, feeding on bacteria, yeasts and algae in the environment. They are facultative parasites, representing a transitional stage between free life and parasitism.

Naegleria fowleri infects healthy individuals, causing a swiftly fatal meningoencephalitis. Amoebae in their active stages enter the nose by inhalation of contaminated, warm, fresh water. The amoebae penetrate the epithelium at the back of the nose and travel along the olfactory nerves into the brain. Infection spreads over the meninges and along the perivascular channels within the substance of the brain.

Naeglerias are amoebo-flagellates, with a transient, non-feeding biflagellate stage in their life-cycle. This is thought to provide in nature for the rapid spread of the organism to fresh pools after rain. This flagellate stage can be induced to form in cultures by diluting the medium with a small amount of distilled water. In common with many other amoebae there is also a resistant cystic stage. Cysts will survive a cold winter if kept moist, but are killed rapidly by desiccation. The feeding stage (trophozoite) has a lobose pseudopodium, used for locomotion. This is generally rapid, in the region of two body lengths per minute. The cell nucleus has a large central body (nucleolus) composed of ribonucleoprotein, and scanty peripheral DNA. The three morphological forms of *N. fowleri* are shown in Fig. 6.1. Multiplication is by binary fission, with a generation time of the order 6–8 hours. Populations grow rapidly by comparison with other protozoa, but more slowly than bacteria.



(a)

(b)

(c)

Fig. 6.1 The three morphological forms of *Naegleria fowleri*: (a) trophozoite (motile vegetative amoeba); (b) cyst; (c) flagellate.

The role of free-living amoebae in primary amoebic meningoencephalitis (PAM) was first recognized in 1965 by Fowler & Carter. At first the pathogen was confused with the ubiquitous *N. gruberi*, but distinguishing features were soon recognized, including preference for warm waters, ability to grow at 45°C (113°F), cyst morphology (size, number of pores) and pathogenicity for mice (*N. gruberi* is non-pathogenic). In 1970 the pathogen was given the trivial names *fowleri* and *aerobia*, the former achieving publication priority by a short head. Differentiation between the two species is now much strengthened by biochemical studies. Indeed *N. gruberi* is now recognized to be a species complex. More than 150 cases of PAM have been reported in the world literature. Only two patients with fully authenticated infections have survived. Although clinical isolates from all over the world are apparently identical serologically, restriction endonuclease digests of nuclear DNA indicate the existence of several genetically distinct lineages from different geographical locations (De Jonckheere 1987). The isolation of *N. fowleri* from the environment has proved difficult. Incubation of samples at elevated temperatures was tried early, with some success. A major source of confusion was the unsuspected existence of a second thermophilic species of *Naegleria*, morphologically and serologically very similar to *N. fowleri*, sharing the same econiche, but non-pathogenic for mice. Not until 1980 was this recognized to be a new species and given the specific name *lovaniensis* by Stevens and her colleagues. The fact that cases of PAM the world over occur in clusters, and that in several instances a succession of fatalities has persisted until measures were taken to eliminate the common source of infection, makes it mandatory to search for the source of infection whenever a case is encountered. Particularly striking was the series of 16 deaths recorded at Usti in Czechoslovakia over a period of four years.

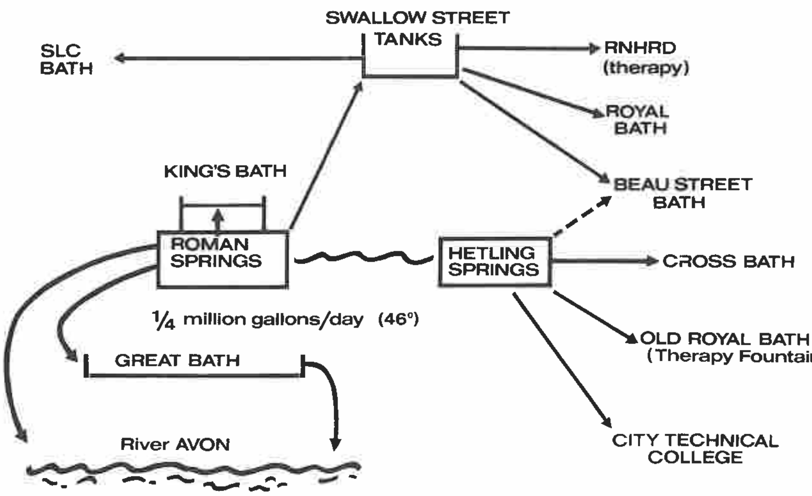


Fig. 6.2 Distribution of spa water from thermal springs to recreational baths and other sites in Bath.

In 1978 in Bath we had first to consider as possible sources a small swimming pool and a fish-tank at the child's school. Both proved negative. Proceeding then to the Beau Street baths, where the school held a swimming gala shortly before the child fell ill, samples from a holding tank above the former spa laundry in Swallow Street yielded a *Naegleria* amoeba which grew at the same high temperature as the CSF isolate, readily produced a flagellate phase, and reacted in the indirect fluorescent antibody test with anti-*N. fowleri* serum at the same titre as the CSF isolate. It now became imperative to examine the Roman springs beneath the King's Bath. This necessitated draining the bath and providing lighting and ventilation for the enclosed space where the springs rise. Samples yielded several more apparently identical *Naegleria* isolates. This evidence was sufficient at that time to identify the organism as *Naegleria fowleri*. In view of the association with a fatal case of PAM it was necessary to recommend the closure of the thermal springs and of the baths they served (Fig. 6.2).

Access to the Roman springs for our amoebal investigation also revealed to the City Engineers that the foundations of the Pump Room were being severely eroded. Substantial underpinning of the buildings and measures to avoid further erosion were seen to be necessary. To facilitate this work it was decided to cut away the concrete floor placed under the King's Bath by Major Davies at the end of the 19th Century. This in turn provided an opportunity, magnificently seized, to excavate the Roman temple remains known to be lying beneath the Pump Room and Abbey Churchyard (Cunliffe 1971).

The description in 1980 of *N. lovaniensis* as a new thermophilic, non-pathogenic *Naegleria*, distinct from *N. fowleri*, necessitated a reappraisal of our 1978 environmental isolates. Development of a cellulose acetate isoenzyme electrophoresis system for naeglerias enabled us to show that glucose phosphate isomerase and phosphoglucomutase provided clear distinctions between 'non-pathogenic' and 'pathogenic' isolates. Moreover Ann Stevens and her colleagues had shown that electron microscopy reveals important structural differences between *N. fowleri* and *N. lovaniensis*. The nucleus of *N. lovaniensis* has around it a wrapping of rough endoplasmic reticulum, which is not present in *N. fowleri*. Use of the new isoenzyme technique and electron microscopy showed that all our 1978 environmental isolates were *N. lovaniensis* and not *N. fowleri*. A fresh hunt for the pathogen was therefore begun in the summer of 1981. By December of the same year *N. fowleri* had been identified in samples from the NE corner of the Great Bath (Fig. 6.3).

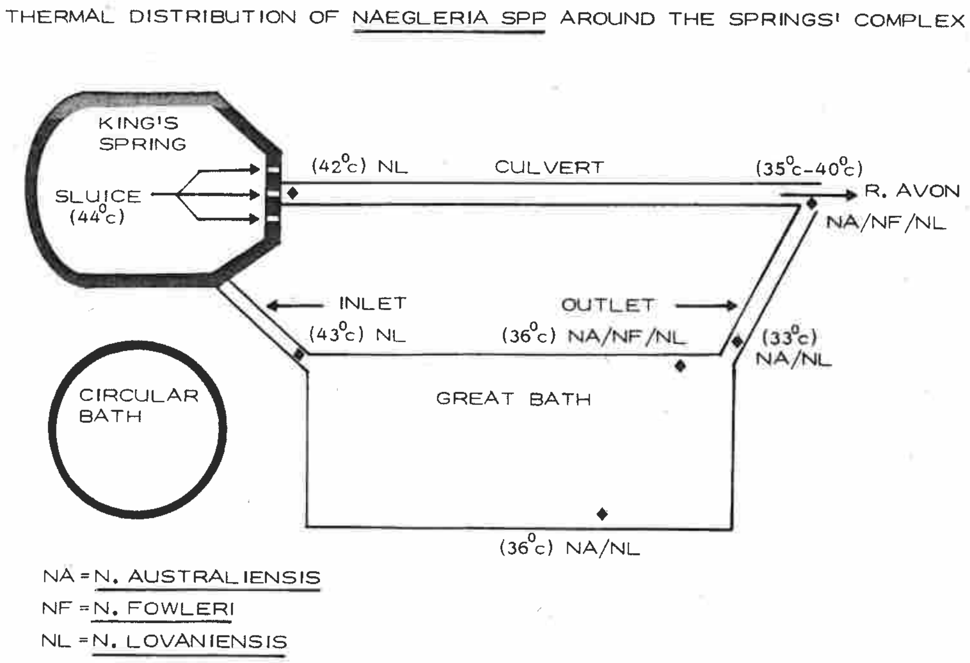


Fig. 6.3 Diagram of Roman baths indicating the location of sites sampled for thermophilic *Naegleriae* 1981–1984.

Identification was carefully validated by electron microscopy, isoenzyme electrophoresis and mouse pathogenicity. Additional enzyme tests since developed in Bath (Kilvington & White 1985) have provided still further confirmation. Further systematic attempts to recover *N. fowleri* from the thermal springs continued, with negative results, throughout 1982 and the first half of 1983. In May of that year sampling was concentrated solely on the point at which the overflow from the Great Bath reaches the main drain into the River Avon. In June 1983 several further fully validated isolations were made.

While the second search for *N. fowleri* was in hand, public demand for a supply of safe spa water for Bath led to a detailed examination of the fault under Bath, up which the Roman thermal springs rise. Dr Geoffrey Kellaway skilfully drove a new oblique borehole to meet the relevant aquifer at a depth of some 60 metres (197 ft), so bringing the water to the surface without contamination by the superficial soil which is the primary home of *Naegleria*. It is unlikely that water derived from such a depth will harbour aerobic amoebae, and prolonged monitoring of the water from its new source has failed to detect any naeglerias to date.

In 1984 a search for a third thermophilic *Naegleria*, *N. australiensis*, was initiated. This species was first definitively described in 1981 by De Jonckheere. Although not yet associated with human disease, it is pathogenic for mice.

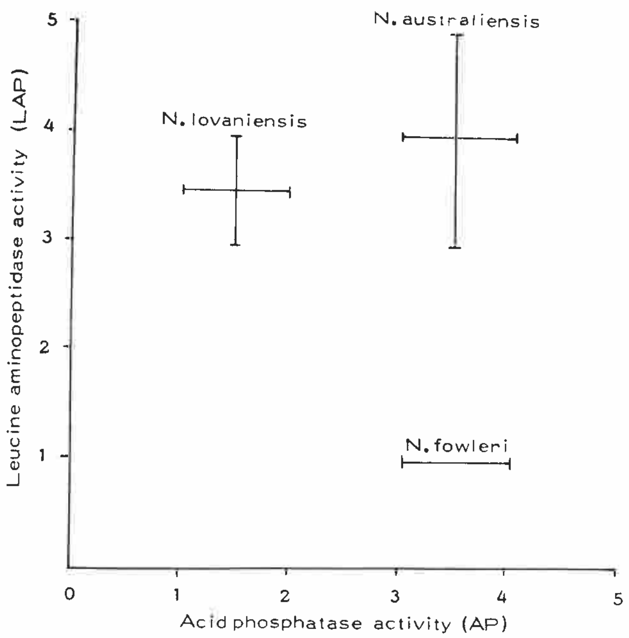


Fig. 6.4 Differentiation between species of thermophilic *Naegleria* by activity patterns for the enzymes Leucine aminopeptidase (LAP) and Acid phosphatase (AP).

The methods previously used to isolate and identify *N. lovaniensis* and *N. fowleri* in the hot springs used incubation at 43/44°C to favour the growth of *N. fowleri*. Since *N. australiensis* will not grow at temperatures above 42°C, cultures were now incubated at 37°C. Primary cultures were made as before on non-nutrient agar supplemented with *Esch. coli*. Plaques of amoebae which resembled *Naegleria* morphologically and produced amoebo-flagellates in distilled water were subcultured in liquid TRIS-buffer/*Esch. coli* medium. After incubation for 48–72 hours the medium was tested for *N. australiensis* antigen by staphylococcal coagglutination (Kilvington & White 1986). Antigen positive cultures were transferred to NNA/*Esch. coli* medium. Amoebal growth on this medium was tested by API–ZYM for production of acid phosphatase and leucine aminopeptidase. Distinctively different patterns of activity for these two enzymes are produced by *N. fowleri*, *N. australiensis* and *N. lovaniensis* (Fig. 6.4, Kilvington & White 1985). Candidate strains of *N. australiensis* selected in this way were then tested by cellulose acetate isoenzyme electrophoresis for glucose phosphate isomerase. Strains identified by this test as *N. australiensis* were tested for mouse-pathogenicity by intracerebral inoculation. Brains taken from dead or moribund mice were examined microscopically for *N. australiensis* after specific immuno-peroxidase staining. Amoebae were also re-isolated from brain-tissue by culture and confirmed as *N. australiensis* by immuno-fluorescence and GPI isoenzyme production. By these means *N. australiensis* was isolated on a number of occasions and from several sites during 1985. These were the first isolations of this *Naegleria* from environmental material reported from the United Kingdom.

In 1986 further inquiry was made into the distribution and relative numbers of the three thermophilic naeglerias in the hot springs system, with particular regard to the temperatures prevailing at the sampling sites. Three sites were examined: the waterfall pool at the foot of the overflow from the King's Spring, the sluice-gate at the NE corner of the Great Bath and the overflow from the Great Bath at its junction with the main drain to the River Avon (Fig. 6.3). Samples of mud were inoculated onto NNA/*Esch. coli* medium and incubated at 37°C. Presumptive *Naegleria* isolates, selected by morphology and flagellate formation, were then tested for specific soluble naegleria antigen by staphylococcal coagglutination. The results of this survey are shown in Figs 6.3 & 6.5.

The waterfall pool yielded *N. lovaniensis* only, in numbers that were constant through the year. The water temperature at this site is always between 42° and 43°C. This may account for the absence of *N. australiensis*, which will not grow at temperatures above 42°C. *N. lovaniensis* and *N. australiensis* were both found at the NE corner of the Great Bath, where temperatures ranged from 29–34°C. The latter species was present only from April onwards, but was dominant during the summer months. Water temperatures at the outlet from the Great Bath to the main drain ranged from 30 to 41°C. Only *N. lovaniensis* was found here, the numbers being highest from July to September.

N. fowleri was not detected on any occasion at any of the three sites throughout the whole 1986 survey. The samples taken during the initial search for *N. australiensis* in 1984/85 had also been negative for *N. fowleri*, as were all the samples taken between January 1982 and June 1983. Impressed by the infrequency of isolations of *N. fowleri*, efforts were directed to devising a selective medium that would permit the growth of this species, while inhibiting *N. lovaniensis* and *N. australiensis*. After trial of a series of diamidines, a suitably selective medium was achieved by incorporating M&B 800A (pentamidine isethionate). In February 1987 this medium was inoculated with samples from the hot springs system. Two strains of *N. fowleri* were recovered from the NE corner of the Great Bath. Duplicate samples examined with the non-selective NNA/*Esch. coli* medium yielded only numerous isolates of *N. lovaniensis* and *N. australiensis*. It seems that the apparent infrequency of *N. fowleri* may have been due to the failure of non-selective media to detect small numbers of *N. fowleri* in the presence of much larger numbers of other thermophilic naeglerias.

In summary, investigations into the tragic death of a child established that the cause was a small free-living amoeba, *N. fowleri*. Examination of the hot springs revealed the presence of three species of thermophilic *Naegleria*, not previously reported from environmental sources in the United Kingdom, viz. *N. fowleri*, *N. lovaniensis* and *N. australiensis*. Local variations in temperature affect the distribution of these amoebae. The hitherto apparent infrequency of *N. fowleri* relates, at least in part, to the difficulty of finding this

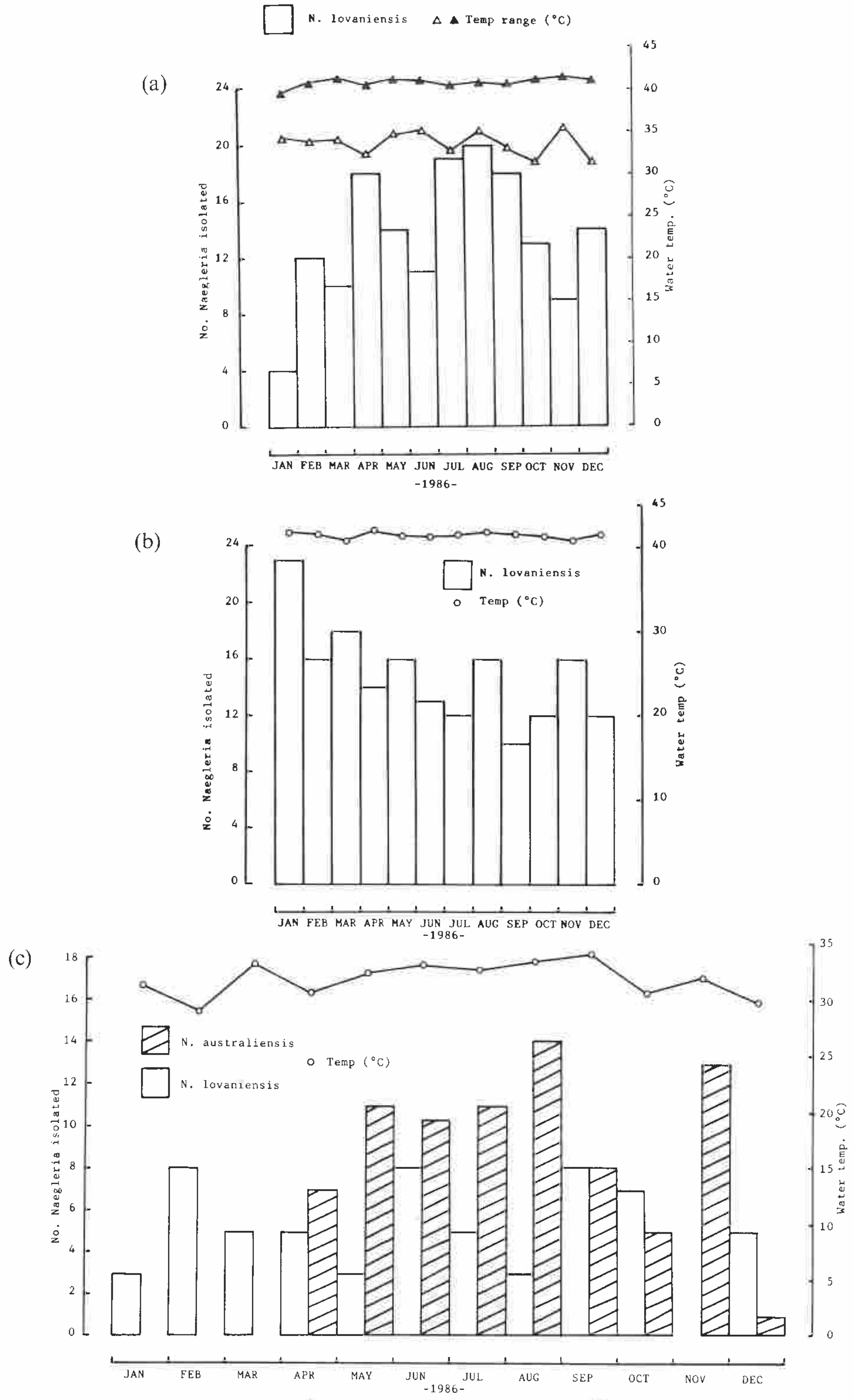


Fig. 6.5 Seasonal variation in relative numbers of thermophilic *Naegleria* by site and temperature (1986) survey) (see also Fig. 3): (a) outlet from Great Bath to Roman drain; (b) pool below waterfall-outlet from King's Spring; (c) outlet from Great Bath to sluice leading to Roman drain.

amoeba when in a minority among larger numbers of the other two thermophils. This may also contribute to the rarity of infection from a source which is probably constantly positive for *N. fowleri*, but generally in small numbers only.

The need to search for a source of infection following a case of PAM had some surprisingly far-ranging consequences for Bath. In searching for amoebae in the Roman springs, erosion of the Pump Room foundations was revealed. Repair work gave an opportunity to uncover the remains of an important Roman temple-complex. Public concern that safe spa water be once more available led to hydrogeological investigation of the system of faults beneath Bath. This provided the basis for sinking a new borehole to the deep aquifer which supplies the healing waters of Bath, thus avoiding the potentially infected spring-pipe which was their original source. The importance of protecting this new source from amoebal re-contamination called for a degree of hygienic care unusual in most water-extraction operations. It is to be hoped that a new range of safe, contemporary spa facilities may yet arise on the site of our earlier establishments.

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