TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE, VOL. 77, NO. 3, 372-377 (1983)

The epidemiology and control of primary amoebic meningoencephalitis with particular reference to South Australia

MARGARET M. DORSCH[1], A. SCOTT CAMERON[2] AND BRET S. ROBINSON[3]

[1]*Epidemiology Branch and* [2]*Communicable Disease Control, South Australia Health Commission;* [3]*Amoeba Research Unit, State Water Laboratories, Engineering and Water Supply Department, South Australia*

Summary

This paper reviews the history, epidemiology and control of primary amoebic meningoencephalitis (PAM), caused by *Naegleria fowleri*, with particular reference to South Australia. The intention has been to outline misconceptions and uncertainties pervading the earlier literature. Although PAM infections elsewhere have been attributed to cysts in air-borne dust, we believe that contact with water in the domestic environment was not adequately considered as a potential source of these infections. Several reports have cast doubt on the effectiveness of chlorine in controlling *N. fowleri*, although there is laboratory and South Australian field experience to the contrary. These reports can be traced to a misunderstanding of the circumstances surrounding cases of PAM reported by other workers. Provided that a continuous free chlorine residual of 0·5 mg/l can be maintained in water accessible to *N. fowleri*, the risk of disease should be negligible. The failure of amphotericin B therapy to save recent victims of the disease, despite relatively prompt intervention, is disappointing. Possible reasons for this, and the reports that rifampin or tetracycline combined with amphotericin might be more successful, are discussed.

Introduction

Between 1955 and 1972, 13 confirmed or very probable cases of primary amoebic meningoencephalitis (PAM) occurred in the Spencer Gulf region of South Australia. Continuous chlorination of water supplies to those towns in which PAM had been reported (Port Augusta, Kadina and Port Pirie) commenced in 1972, subsequent to the isolation of the causal amoebae from a tap at the home of a Port Augusta victim. Nine years later, the first case of PAM at Whyalla was reported during one of the hottest summers on record in South Australia. Before 1980 the only known case from another Australian State was a Queensland boy, who contracted the disease in October 1971. He is the only Australian survivor of PAM. However, in January, 1980, two cases were identified in Western Australia, and an additional victim (from 1963) was diagnosed retrospectively from autopsy records. A further case, the first in New South Wales, was recorded early in 1981 (Table I).

Because four cases of PAM have presented in three Australian states over the last two years, there is once more a need to draw this rare disease to the attention of local clinicians and public health authorities. Furthermore, it seems timely to identify some of the uncertainties and misconceptions which pervade the earlier literature, and to inform a wider audience of the South Australian experience with the disease and its control.

This paper presents: (i) a brief history of PAM; (ii) a summary of the epidemiology of PAM in Australia; and (iii) an outline of the preventive and control measures, particularly those adopted in South Australia.

History

The first formal account of PAM described four South Australian cases (FOWLER & CARTER, 1965). In 1970, CARTER demonstrated that the causative amoeba was a previously undescribed species of *Naegleria*, which he named *N. fowleri*. Shortly beforehand he had also demonstrated both the *in vitro* and *in vivo* susceptibility of this organism to amphotericin B (CARTER, 1972).

In the treatment of PAM, amphotericin B is still regarded as the drug of choice (LEE *et al.*, 1979; STEVENS *et al.*, 1981). However, its failure to arrest the inexorable course of the disease in three recent cases, despite relatively prompt intervention and intrathecal administration, is disappointing (CAIN *et al.*, 1981; STEVENS *et al.*, 1981).

Amphotericin B was administered intrathecally and intraventricularly to the two confirmed survivors of PAM, the more recent California patient receiving this drug in combination with miconazole and rifampin (SEIDEL *et al.*, 1982). Accordingly, it has been postulated that the latter drugs may be useful in treating human *Naegleria* infections (CDC, 1978; THONG *et al.*, 1979a; THONG, 1982).

The experimental studies of THONG *et al.* (1979b) demonstrated a marked synergism between amphotericin B and rifampin *in vivo*. However, STEVENS *et al.* (1981) could not replicate this additive effect in mice challenged with *N. fowleri* isolated from one of their patients. This result suggests that there may be inter-strain differences in drug sensitivity. The likely efficacy of miconazole in human cases is also equivocal. *In vitro*, miconazole used alone has exhibited activity against *N. fowleri*, and the combination of amphotericin B and miconazole is reported to have a synergistic effect (SEIDEL, 1982). *In vivo* studies with this drug combination, using the murine model, have failed to show a protective effect. However, this may

Address for reprints: Dr. A. S. Cameron, Communicable Disease Control Unit, S.A. Health Commission, G.P.O. Box 1313, Adelaide, South Australia 50001.

Table I—Details of Australian PAM Cases 1955-81 in chronological order

Place	State	Age	Sex	Died (date)	Diagnosis confirmed	Amphotericin B	Comments (References)
Pt. Pirie	SA	10	F	15/02/55	No	No	Retrospective diagnosis. Amoebae seen in wet preparations of CSF. (DORSCH, 1982)
Pt. Augusta	SA	7	M	31/01/61	Yes	No	No recorded history of swimming in freshwater. (FOWLER & CARTER, 1965; CARTER, 1970)
Merredin	WA	4	M	–/02/63	Yes	No	Retrospective diagnosis. Autopsy findings and specific immunofluorescence studies of brain sections consistent with *N. fowleri*. No recorded history of swimming. (MILLER *et al.*, 1982)
Pt. Augusta	SA	37	M	11/03/63	Yes	No	Non-swimmer. (CARTER, 1970)
Pt. Augusta	SA	9	F	04/02/65	Yes	No	No recorded history of swimming in freshwater. (FOWLER & CARTER, 1965)
Kadina	SA	8	F	26/02/65	Yes	No	History of immersing head in school water trough and playing in bath. (FOWLER & CARTER, 1965; DORSCH, 1982)
Pt. Augusta	SA	28	M	12/03/65	Yes	No	Non-swimmer. No other recorded history of intranasal contact with freshwater. (FOWLER & CARTER, 1965)
Pt. Augusta	SA	10	M	11/01/66	Yes	No	No recorded history of intra-nasal contact with freshwater. (CARTER, 1970)
Kadina	SA	8	M	26/01/69	Yes	Yes	History of swimming in open-air pools. (CARTER, 1970)
Pt. Augusta	SA	8	F	14/02/70	Yes	No	No recorded history of swimming or other intra-nasal contact with freshwater. (CARTER, 1972)
Pt. Pirie	SA	26	F	25/02/71	No	No	Retrospective diagnosis on basis of: (i) close association in time and place with subsequent confirmed victim; (ii) rapidly fatal clinical course; and (iii) history of "washing out nose" under shower. (DORSCH, 1982)
Pt. Pirie	SA	16	M	27/02/71	Yes	Yes	Swam frequently in open-air pools. (CARTER, 1972)
Mt. Morgan	QLD	14	M	Survived Onset: 21/10/71	Yes	Yes	Amoebae cultured from CSF were pathogenic in mice, and appear to be typical *N. fowleri* on morphological, serological and enzyme electrophoresis criteria. ATCC No. 30463. Swam in open-air pool. (ANDERSON & JAMIESON, 1972; CARTER, 1972; DORSCH, 1982)
Pt. Augusta	SA	7	F	06/02/72	Yes	Yes	History of swimming in public pool and backyard pools (unfiltered, unchlorinated). *N. fowleri* isolated from tap water at the home of a concurrent case. (ANDERSON & JAMIESON, 1972)
Pt. Augusta	SA	5	M	10/02/72	Yes	Yes	History of "playing submarines" in bath. *N. fowleri* isolated from backyard tap and other locations in Pt. Augusta soon afterwards (ANDERSON & JAMIESON, 1972)
Beverley	WA	11	F	–/01/80	Yes	No	Diagnosis of PAM not suspected during life. Swam in public and backyard pools. *Naegleria* spp. isolated from public pool mains supply and backwash sump. (COMMONWEALTH DEPT. HEALTH, 1980; MILLER *et al.*, 1982)
Merredin	WA	7	M	–/01/80	Yes	Yes	History of swimming in backyard pools. (COMMONWEALTH DEPT. HEALTH, 1980; MILLER *et al.*, 1982)
Whyalla	SA	10	M	28/01/81	Yes	Yes	History of swimming in open-air public pool. *N. fowleri* isolated from mains water samples soon after. Booster chlorination subsequently introduced for Whyalla. (DORSCH, 1982)
Richmond	NSW	3	M	03/02/81	Yes	No	PAM not suspected prior to autopsy. History of swimming in open-air pool. (COMMONWEALTH DEPT. HEALTH, 1981)

be due to poor penetration into the cerebrospinal fluid in mice after oral or intraperitoneal administration (THONG *et al.*, 1979a). Lack of access to the site of infection by this drug combination may, in part, explain the unsuccessful outcome of treatment in the first case described by STEVENS *et al.* (1981).

The efficacy of a simple combination of rifampin and amphotericin B in human victims is as yet untried, as is the combination of amphotericin and tetracycline, which has shown very promising results both *in vitro* (LEE *et al.*, 1979) and *in vivo* (THONG *et al.*, 1979b). STEVENS *et al.* (1981) have suggested that the intra-cisternal route of therapy might facilitate more direct and rapid access to sites of infection. Likewise, this has yet to be tested in the clinical setting.

Given that several U.S. patients since 1978 have been treated with the same drug regimen as the California survivor (although not under entirely comparable circumstances) without success, it appears that the issue of appropriate therapy is still far from resolved (SEIDEL, 1982). None the less, recent discussions on the subject have all emphasized the importance of early diagnosis, combined with careful intensive care management and aggressive chemotherapy, if there is to be any hope of success.

Virtually all the reported cases of PAM, with the notable exception of some of those occurring in South Australia, have followed swimming in fresh water—in semi-natural lakes, heated indoor pools, thermally polluted streams, or geothermal springs. Australia is unique in that public water supplies (piped overland, sometimes for hundreds of kilometres) may have been the ultimate source of the pathogen in most cases (Fig. 1). Notably, the first isolation of *N. fowleri* from the environment was that made by ANDERSON & JAMIESON (1972) at Port Augusta.

It was once widely believed that infection could occur as the result of inhalation of air-borne cysts. However, experimental work has shown that cysts of *N. fowleri* cannot survive desiccation lasting more than five minutes (CHANG, 1978). Several PAM infections in Nigeria have been attributed to cysts in air-borne dust (LAWANDE *et al.*, 1979), but we believe that contact with water in the domestic environment was not adequately considered as a potential source of these infections.

The official response to outbreaks of PAM in other countries has generally been to close suspected sources of infection (temporarily or permanently) and/or to warn swimmers of the potential danger. In some cases, disinfection of water was begun or existing disinfection practices were modified. In South Australia, those northern public water supplies known to be contaminated by *N. fowleri* are disinfected by continuous chlorination. Educational programmes to increase public awareness of PAM have been used in South Australia every summer since 1972.

Epidemiology: Australian cases

Of the 19 cases of PAM recorded in Australia, 14 occurred in South Australia, three in Western Australia, and one each in Queensland and New South Wales (Table I). It is possible there have been other unrecognized cases.

The Queensland case has never been fully described in the literature (ANDERSON & JAMIESON, 1972; CARTER, 1972). Consequently, one reviewer has expressed doubts about the validity of the diagnosis and, by implication, the boy's cure (GRIFFIN, 1978). Amoebae cultured from this patient's CSF were pathogenic in mice, and on the basis of morphological, serological and zymogram criteria, are typical *N. fowleri*. The isolate is lodged with the American Type Culture Collection (ATCC 30463). When this patient was last seen in hospital for routine blood tests in 1977, he was reported to be in good health with no evidence of neurological deficit. Internationally, only one other confirmed case, a nine-year-old California girl, is reported to have recovered after infection by *N. fowleri* (CENTERS FOR DISEASE CONTROL, 1978).

Whether the two confirmed survivors of PAM possessed greater natural immunity than other victims is still a matter for conjecture. CURSONS *et al.* (1977) have suggested that prior exposure to the ubiquitous, non-pathogenic *N. gruberi* may confer immunity against the less widespread but pathogenic *N. fowleri*. The more recently described species, *N. lovaniensis* (STEVENS *et al.*, 1980), may be even more important in this respect because of its greater antigenic similarity to *N. fowleri*.

Between February, 1972 and January, 1981 there were no PAM cases in South Australia. Before the discovery of *N. fowleri* in the Morgan-Whyalla pipeline and the commencement of continuous chlorination, there had been sporadic cases at frequent intervals, with the highest incidence in Port Augusta (Table I).

For many years the observed clustering of cases in the Spencer Gulf region (Fig. 1) defied explanation. Moreover, the fact that nearly half these victims had not been swimming in fresh water in the week before their illness was a unique and puzzling feature of the epidemiology of PAM in this State. Such a history had been the epidemiological link between all overseas cases of the disease. This apparent anomaly was not resolved until the pathogen was isolated from reticulated water at Port Augusta. Since some of those South Australian cases who had not been swimming did have a specific history of intra-nasal contact with fresh water (e.g., playing in the domestic bath, or "washing out" the nose), it was concluded that public water supplies could have been a source of the pathogen (Table I).

Interestingly, the situation in Western Australia parallels that of South Australia, in that the inland towns of Merredin and Beverley (Fig. 1) are both served by overland pipelines.

In the Queensland and New South Wales cases, open air swimming pools were implicated as possible sources of the infecting organisms (ANDERSON & JAMIESON, 1972; COMMONWEALTH DEPARTMENT OF HEALTH, 1981).

The distribution of Australian cases by month of occurrence is shown in Fig. 2. Apparently, outbreaks of PAM are dependent upon seasonal factors because all cases have occurred in the summer months, between October and March. Most, but by no means all, of the Australian cases have been associated with heat wave conditions.

Current evidence and experience suggest that the

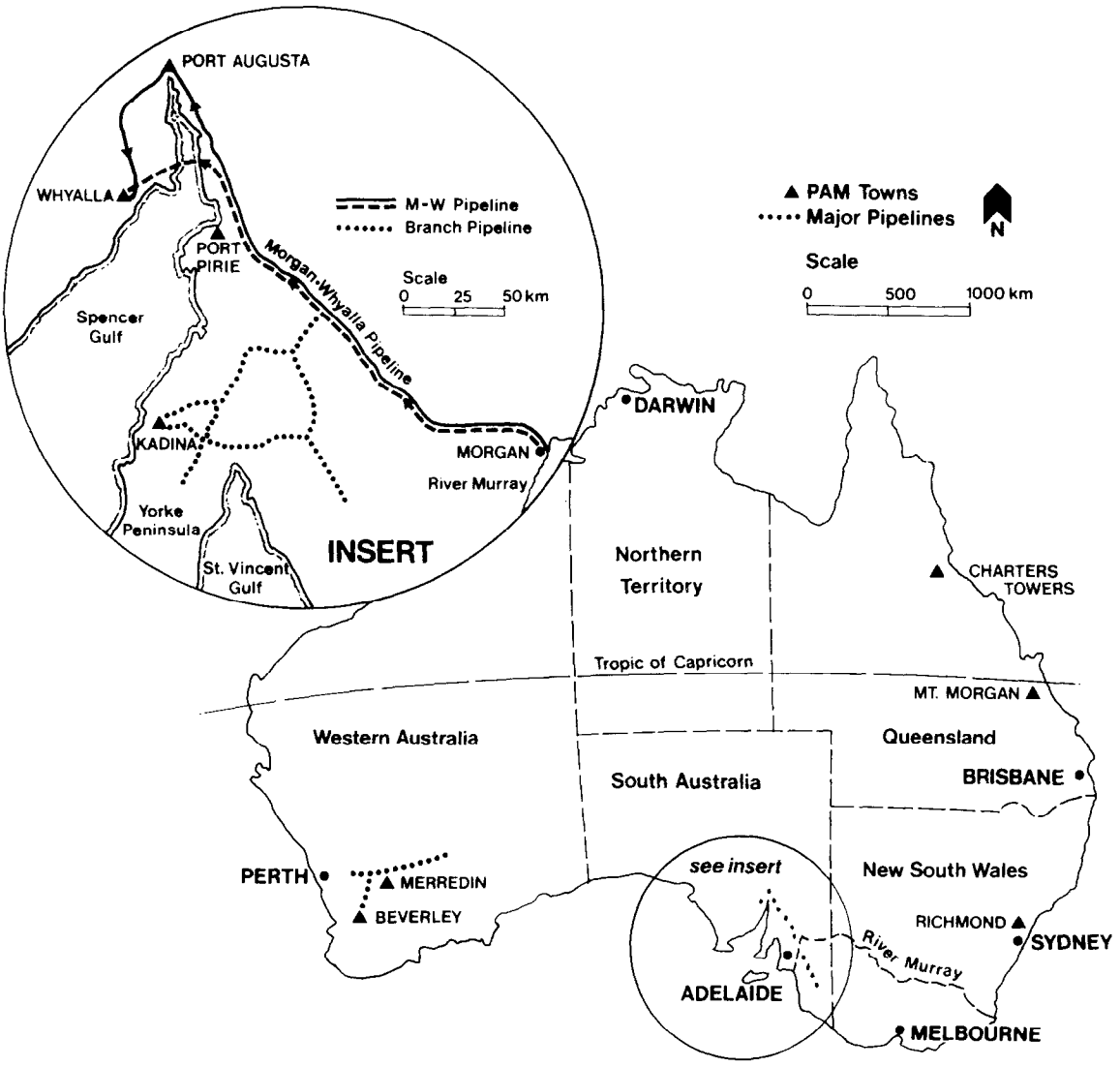

Fig. 1. Map of Australia showing towns in which cases of PAM have occurred and water supply pipelines of epidemiological significance.

summer months constitute a period of high risk for PAM because: (i) water-related activities are more frequent or prolonged at this time; and (ii) summer water conditions favour growth and multiplication of the pathogen.

N. fowleri requires high water temperatures to multiply and compete successfully with other amoebae (GRIFFIN, 1972). In South Australia, results of the State Water Laboratories' weekly amoeba monitoring programme have shown a seasonal variation in the frequency at which *N. fowleri* is isolated from public water supplies (ROBINSON & LAKE, 1981).

16 of the total of 19 Australian cases (i.e., 80%) were less than 15 years old, and males outnumbered females by almost two to one (12M: 7F). This pattern of occurence is generally accepted as being consistent with the greater frequency and duration of boisterous activities around water in these groups.

Control of *Naegleria fowleri* by chlorination

Experimental studies have demonstrated that cysts and trophozoites of *N. fowleri* are killed by free chlorine (DERREMAUX *et al.*, 1974; DE JONCKHEERE & VAN DE VOORDE, 1976; ROBINSON, 1977; CHANG, 1978; CURSONS *et al.*, 1980). However, several reports in the literature cast doubt on the effectiveness of chlorine in controlling *N. fowleri* in practice. This view has been perpetuated in authoritative text books on communicable disease control.

ANDERSON & JAMIESON (1972) described the water supplied to Port Augusta as "unfiltered, chlorinated river water". Until their isolation of *N. fowleri* from this supply, however, water from the River Murray at

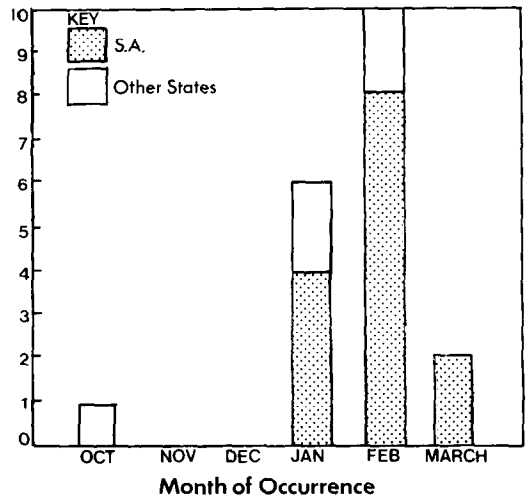

Fig. 2. Seasonal distribution of Australian cases of PAM.

Morgan was only chlorinated for brief periods each week to prevent fouling of the pipeline (WALTERS *et al.*, 1981). Since the water is pumped 280 km from the source, it is unlikely that a free chlorine residual ever persisted as far as Port Augusta. Subsequent installation of a number of permanent chlorination stations in the Morgan-Whyalla system has led to satisfactory control of *Naegleria* species in water supplied to towns where cases of PAM have occurred (ROBINSON, 1977; WALTERS *et al.*, 1981).

In the latter studies, *Naegleria* species were occasionally isolated from locations where chlorine levels were very low. Most of these isolates were non-pathogenic strains. Since *N. fowleri* is a free-living amoeba, it may multiply under favourable conditions to densities which could lead to infections, even though the numbers surviving disinfection are too low to be detected in any sampling programme. Continuity and persistence of disinfection are clearly important in controlling *N. fowleri*.

ANDERSON & JAMIESON (1972) also described a swimming pool in which *Naegleria* species persisted despite a chlorine dose of 10 mg/l. However, the chlorine demand of swimming pool water is highly variable (WARREN & RIDGWAY, 1978). Since chlorine residuals were not reported by Anderson and Jamieson, it is possible that the chlorine demand was not satisfied by this dose.

The best documented example of contamination of a swimming pool by *N. fowleri* is the Czechoslovakian pool responsible for 16 cases of PAM. CERVA (1971) showed that contamination of the pool by various amoebae occurred despite maintenance of 0·3 mg/l free chlorine residual, and that a 10-fold increase in the density of amoebae occurred as water passed through the swimming pool filter. A later study revealed a water-filled cavity behind a cracked wall of the pool, in which *N. fowleri* and other amoebae were growing relatively inaccessible to chlorine (KADLEC *et al.*, 1980). LYONS & KAPUR (1977) reported amoeba densities of 2,800 to above 10,000 per litre in backwash water from a swimming pool filter. Protection of micro-organisms from chlorine in the filter material and conditions which allow their release into the pool have been discussed in detail by WARREN & RIDGWAY (1978).

It is apparent that most situations in which chlorination was unsuccessful in controlling *N. fowleri* can be explained either by: (i) the existence of conditions which allow *N. fowleri* to survive, inaccessible to the chlorine; or (ii) the failure of the persistence or continuity of disinfection.

Conclusions

PAM is a rare, but rapidly fatal disease of previously healthy young persons, caused by the amoeba *N. fowleri*. Although a single case of meningoencephalitis apparently caused by *N. gruberi* has been reported from the UK (APLEY *et al.*, 1970; SAYGI *et al.*, 1973), we believe the evidence for this is weak. The amoebae originally isolated from this patient could conceivably have been a mixed culture of *N. fowleri* and *N. gruberi*, of which only the *N. gruberi* survived during subsequent laboratory culture.

Only two confirmed victims of *N. fowleri* are known to have survived. Intrathecal and intraventricular amphotericin B was administered in both instances, with one patient also receiving miconazole and rifampin. *In vivo* studies have demonstrated synergism between amphotericin and rifampin (THONG *et al.*, 1979b). Similarly, experimental work with a combination of tetracycline and amphotericin has produced promising results (LEE *et al.*, 1979; THONG *et al.*, 1979a). Simple combinations of these two drugs, and the intra-cisternal route of therapy proposed by STEVENS *et al.* (1981), may offer greater hope for successful treatment of future victims.

Given that cysts of *N. fowleri* cannot survive desiccation lasting more than five minutes (CHANG, 1978), we find the hypothesis of infection through cysts carried in air-borne dust to be untenable. Our knowledge of the epidemiology of PAM in South Australia leads us to suggest that intimate contact with fresh water in the domestic environment should be considered as a potential source of infection in all cases with no history of recent swimming.

Australia is unique in that public water supply systems, delivering water to inland towns over large distances, may have been the ultimate source of the infective agent in most cases of PAM. However, it is emphasized that the introduction of continuous chlorination to South Australia's northern water supplies, to achieve a free chlorine residual of 0·5 mg/l at the point of delivery to consumers, has led to: (i) effective control of *Naegleria* species in those supplies; and (ii) the apparent elimination of PAM from towns where it was previously thought to be endemic. The South Australian experience with chlorination over 10 years in the field therefore vindicates experimental findings that cysts and trophozoites of *N. fowleri* are killed by free chlorine. It is concluded that circumstances in which chlorination was apparently ineffective can be explained by the failure of persistence or continuity of disinfection, and/or by the existence of conditions which limited chlorine access to sites of *N. fowleri* growth.

Addendum

Since the submission of this paper, the State Water Laboratories, South Australia, have confirmed the

diagnosis of PAM in a case referred by Dr. D. Symes, Commonwealth Health Laboratory, Cairns, Queensland. The case was a 10-month-old male from Charters Towers (see Fig. 1) who died on 3rd December, 1982. Possible sources of infection, including a small wading pool and a backwater of the Burdekin River, are still under investigation.

References

Anderson, K. & Jamieson, A. (1972). Primary amoebic meningoencephalitis. *Lancet*, **1**, 902-903.

Apley, J., Clarke, S. K. R., Roome, A. P. C. H., Sandry, S. A., Saygi, G., Silk, B. & Warhurst, D. C. (1970). Primary amoebic meningoencephalitis in Britain. *British Medical Journal*, **i**, 596-599.

Cain, A. R. R., Wiley, P. F., Brownell, B. & Warhurst, D. C. (1981). Primary amoebic meningoencephalitis. *Archives of Disease in Childhood*, **56**, 140-143.

Carter, R. F. (1970). Description of a *Naegleria* sp. isolated from two cases of primary amoebic meningoencephalitis, and of the experimental pathological changes induced by it. *Journal of Pathology*, **10**, 217-244.

Carter, R. F. (1972). Primary amoebic meningoencephalitis: An appraisal of present knowledge. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **66**, 193-213.

Centers for Disease Control (1978). Primary amebic meningoencephalitis California, Florida, New York. *Morbidity and Mortality Weekly Report*, **27**, 343-344.

Cerva, L. (1971). Studies of limax amoebae in a swimming pool. *Hydrobiologia*, **38**, 141-161.

Chang, S. L. (1978). Resistance of pathogenic *Naegleria* to some common physical and chemical agents. *Applied and Environmental Microbiology*, **35**, 368-375.

Commonwealth Department of Health (1980). Primary amoebic meningoencephalitis—Western Australian outbreak. *Communicable Diseases Intelligence*, Bulletin No. 80/3, Canberra.

Commonwealth Department of Health (1981). Primary amoebic meningoencephalitis—New South Wales. *Communicable Diseases Intelligence*, Bulletin No. 81/17, Canberra.

Cursons, R. T. M., Brown, T. J. & Keys, E. A. (1977). Immunity to pathogenic free-living amoebae. *Lancet*, **ii**, 875-876.

Cursons, R. T. M., Brown, T. J. & Keys, E. A. (1980). Effect of disinfection on pathogenic free-living amoebae in axenic conditions. *Applied and Environmental Microbiology*, **40**, 62-66.

De Jonckheere, J. & van de Voorde, H. (1976). Differences in destruction of cysts of pathogenic and nonpathogenic *Naegleria* and *Acanthamoeba* by chlorine. *Applied and Environmental Microbiology*, **31**, 294-297.

Derremaux, A. L., Jadin, J. B., Willaert, E. & Moret, R. (1974). Action du chlore sur les amibes de l'eau. *Annales de la Société Belge de Médecine Tropicale*, **54**, 415-428.

Dorsch, M. M. (1982). Primary Amoebic Meningoencephalitis: An Historical and Epidemiological Perspective with Particular Reference to South Australia. Adelaide: South Australian Health Commission.

Fowler, M. & Carter, R. F. (1965). Acute pyogenic meningitis probably due to *Acanthamoeba* sp: a preliminary report. *British Medical Journal*, **ii**, 740-742.

Griffin, J. L. (1972). Temperature tolerance of pathogenic and nonpathogenic free-living amoebas. *Science*, **178**, 869-870.

Griffin, J. L. (1978). Pathogenic free-living amoebae. In: *Parasitic Protozoa*, Vol. 2, Kreier, J. P. (Editor). New York: Academic Press, pp. 507-549.

Kadlec, V., Skvarova, T., Cerva, L. & Nebazniva, D. (1980). Virulent *Naegleria fowleri* in indoor swimming pool. *Folia Parasitologica*, **27**, 11-17.

Lawande, R. V., John, I., Dobbs, R. H. & Egler, L. J. (1979). A case of primary amebic meningoencephalitis in Zaria, Nigeria. *American Journal of Clinical Pathology*, **71**, 591-594.

Lee, K. K., Karr, S. L., Wong, M. M. & Hoeprich, P. D. (1979). In vitro susceptibilities of *Naegleria fowleri* strain HB-1 to selected antimicrobial agents, singly and in combination. *Antimicrobial Agents and Chemotherapy*, **16**, 217-220.

Lyons, T. B. & Kapur, R. (1977). Limax amoebae in public swimming pools of Albany, Schenectady and Rensselaer Counties, New York: their concentration, correlations and significance. *Applied and Environmental Microbiology*, **33**, 551-555.

Miller, G., Cullity, G., Walpole, I., O'Connor, J. & Masters, P. (1982). Primary amoebic meningoencephalitis in Western Australia. *Medical Journal of Australia*, **i**, 352-357.

Robinson, B. S. (1977). Effectiveness of chlorine in the control of *Naegleria:* laboratory and field studies in South Australia. *Proceedings of the 7th Federal Convention of the Australian Water and Wastewater Association*, Canberra, 1977, pp. 465-478.

Robinson, B. S. & Lake, J. A. (1981). The influence of temperature on growth and distribution of *Naegleria* species. *Proceedings of 9th Federal Convention of the Australian Water and Wastewater Association*, Perth, 1981, Section 3, pp. 12-19.

Saygi, G., Warhurst, D. C. & Roome, A. P. C. H. (1973). A study of amoebae isolated from the Bristol cases of primary amoebic encephalitis. *Proceedings of the Royal Society of Medicine*, **66**, 277-282.

Seidel, J. (1982). Chemotherapy for primary amebic meningoencephalitis. (Letter). *New England Journal of Medicine*, **306**, 1296.

Seidel, J. S., Harmatz, P., Visvesvara, G. S., Cohen, A., Edwards, J. & Turner, J. (1982). Successful treatment of primary amoebic meningoencephalitis. *New England Journal of Medicine*, **306**, 346-348.

Stevens, A. R., De Jonckheere, J. & Willaert, E. (1980). *Naegleria lovaniensis* new species: isolation and identification of six thermophilic strains of a new species found in association with *Naegleria fowleri*. *International Journal for Parasitology*, **10**, 51-64.

Stevens, A. R., Shulman, S. T., Lansen, T. A., Cichon, M. J. & Willaert, E. (1981). Primary amoebic meningoencephalitis: a report of two cases and antibiotic and immunological studies. *Journal of Infectious Diseases*, **143**, 193-199.

Thong, Y. H. (1982). Chemotherapy for primary amebic meningoencephalitis. (Letter). *New England Journal of Medicine*, **306**, 1295-1296.

Thong, Y. H., Rowan-Kelly, B. & Ferrante, A. (1979a). Delayed treatment of experimental amoebic meningoencephalitis with amphotericin B and tetracycline. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **73**, 336-337.

Thong, Y. H., Rowan-Kelly, B. & Ferrante, A. (1979b). Treatment of experimental *Naegleria* meningoencephalitis with a combination of amphotericin B and rifamycin. *Scandinavian Journal of Infectious Diseases*, **11**, 151-153.

Walters, R. P., Robinson, B. S. & Lake, J. A. (1981). Experiences in the control of *Naegleria* in public water supplies in South Australia. *Proceedings of the 9th Federal Convention of the Australian Water and Wastewater Association*, Perth, 1981, Section 3, pp. 1-11.

Warren, I. C. & Ridgway, J. (1978). Swimming pool disinfection. *Water Research Centre Technical Report TR90*. United Kingdom.

Accepted for publication 29th October 1982.