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EPIDEMIOLOGICAL AND IMMUNOELECTROPHORETIC STUDIES ON HUMAN AND ENVIRONMENTAL STRAINS OF *NAEGLERIA FOWLERI*

by

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Summary — Primary amoebic meningo-encephalitis in South Australia presented a more confusing picture than in other parts of the world because most of the cases showed no history of swimming in fresh water. Nevertheless, one of these *N. fowleri* strains isolated from human and two strains isolated from reticulated water supply showed a complete antigenic identity with human strains isolated from other areas of the world. Strains, that probably can be considered as variants of the human pathogenic species have been isolated in South Australia.

Immunoelectrophoretical analysis is considered as an important technique in the identification and comparison of *N. fowleri* strains.

Introduction

Primary amoebic meningo-encephalitis (PAME) has been recognised as a world-wide fatal human disease caused by amoebae belonging to the genus *Naegleria*. This free-living amoeba has been successively named *N. fowleri* (Carter, 1970), *N. aerobia* (Singh and Das, 1970) and *N. invades* (Chang, 1971).

In most areas of the world the cases of PAME have a history of swimming, nevertheless, in South Australia, there has been a high incidence of cases with no history of swimming (Anderson and Jamieson, 1972). The towns where cases have occurred are separated from each other by a distance of approximately 80 kilometers. They have a most important factor in common, they have no natural water resources, but are supplied with water through the same concrete pipeline which stretches overland from the River Murray and becomes heated during the summer (figure 1). Water samples from taps taken in houses supplied by this pipeline and samples from the pipeline itself have yielded mouse pathogenic *Naegleria*.

These strains have been tested by agglutination and mouse pathogenicity test (Anderson and Jamieson, 1972) and some of them have been compared, by use of immunoelectrophoretic analysis, with human strains isolated all over the world (Willaert *et al.*, 1973).

Contact with water is certainly the main source of infection, but pathogenic strains can survive in dry soil. Recently, in South Australia a mouse pathogenic strain was isolated from dry soil in an arid region.

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In other countries of the world pathogenic *Naegleria* strains have also been isolated.

A retrospective study showed that previously inexplicable fatal cases with no history of swimming in fresh water, had a history of direct introduction of pipeline water into the upper nasal passages.

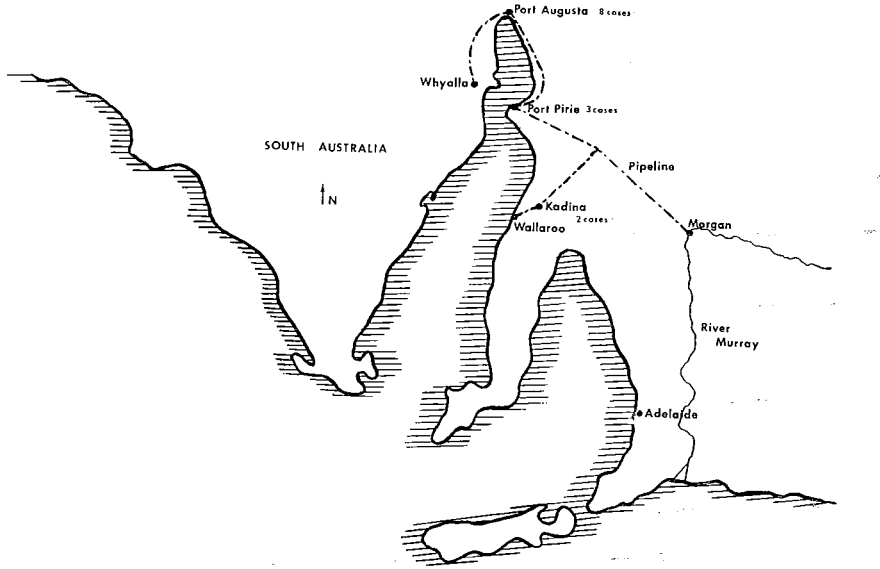


Figure 1

Region of South Australia with the distribution of cases and the trajectory of the pipeline.

Materials and Methods

1. *Isolation, identification and maintenance of amoebae*

Amoebae were isolated from samples of different origin on 1,5 per cent agar plates, spread with a heat-killed suspension of *E. coli*. Cultures were incubated at 28 °C and 37 °C. Morphology and flagellate transformation observed by microscopy, allowed the selection of isolates belonging to the genus *Naegleria*. Axenic cultures of *Naegleria* were obtained in the « A » medium of Fulton (1970) and on medium « CGVS » (Willaert, 1971).

2. *Strains*

2.1. *N. fowleri* of human origin; strains 0 359 and 0 360 (Jadin *et al.*, 1971, Belgium), Vitek (Cervà L., 1969, Czechoslovakia), HB-1 (Butt, 1966, USA), Morgan (Anderson and Jamieson, 1972, Australia) and NH.1 (1972, New Zealand).

2.2. *Naegleria* spp. free-living, mouse pathogenic, strains PA90 and PA117 (Anderson and Jamieson, 1972, Australia) and PAa (Anderson *et al.*, 1973, Australia).

3. *Serological methods*

3.1. Agglutination tests were carried out by the method of Anderson and Jamieson (1972) by mixing a formalinized suspension of axenically growing amoebae with dilutions of an antiserum anti-*N. fowleri.*

3.2 The preparation of antigenic extracts, hyperimmunsera (HIS) and the methods of immunoelectrophoretic analysis (IEA) were executed following the methods described by Biguet *et al.* (1965) applied to amoebae (Willaert *et al.,* 1972).

Results

1. *Human strains*

1.1 Agglutination tests, carried out with an antiserum produced against Carter's original *N. fowleri* strain, confirmed that human isolates originated from America, Czeschoslovakia, New Zealand, Australia and Belgium are identical.

1.2 An hyperimmune antiserum *N. fowleri* 0 359, showing 27 antigenic components in an homologous reaction, was opposed to the hydrosoluble extracts of the strains HB-1 (which served to represent *N. aerobia* and *N. invades*) Vitek, NH.1, Morgan (*N. fowleri*, Australia) and 0 360.

The immunoelectrophoretic analysis, followed by absorption of the HIS by means of the heterologous antigenic extracts, showed completely isologous structures. From this immunostructural identity, the geographical antigenic homogeneity of this species can be established (figure 2).

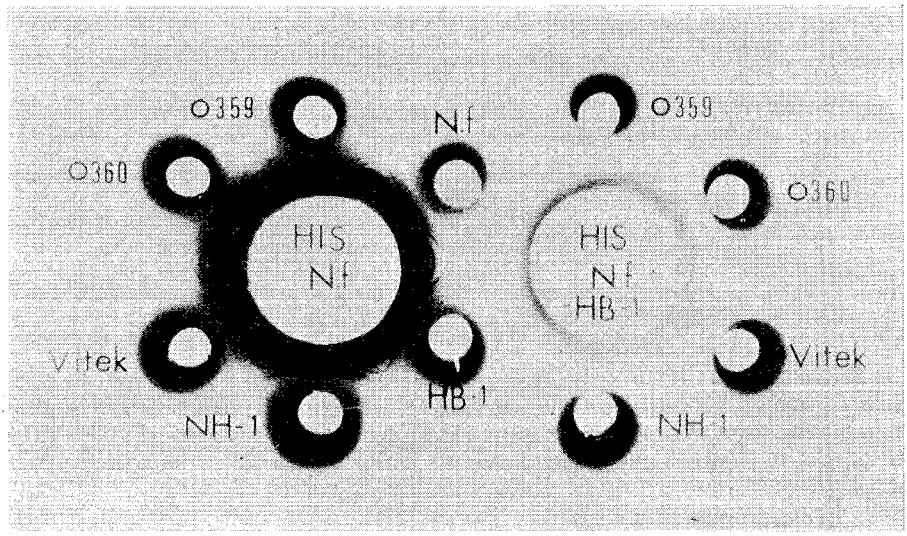


Figure 2

Ouchterlony plate with an hyperimmunserum anti-*N. fowleri* (N. f.) before (central left well) and after absorption (central right well) with strain HB-1, opposed to the strains of *N. fowleri* (Morgan), HB-1, 0 359, 0 360, Vitek and NH-1.

1.3. An HIS anti-*N. fowleri* (Morgan) isolated in Australia, showing 28 antigenic components in an homologous reaction, was opposed to the hydrosoluble extracts of the strains HB-1 and 0 359. The IEA followed by absorption of this HIS with the heterologous extracts showed completely isologous structures (figure 3).

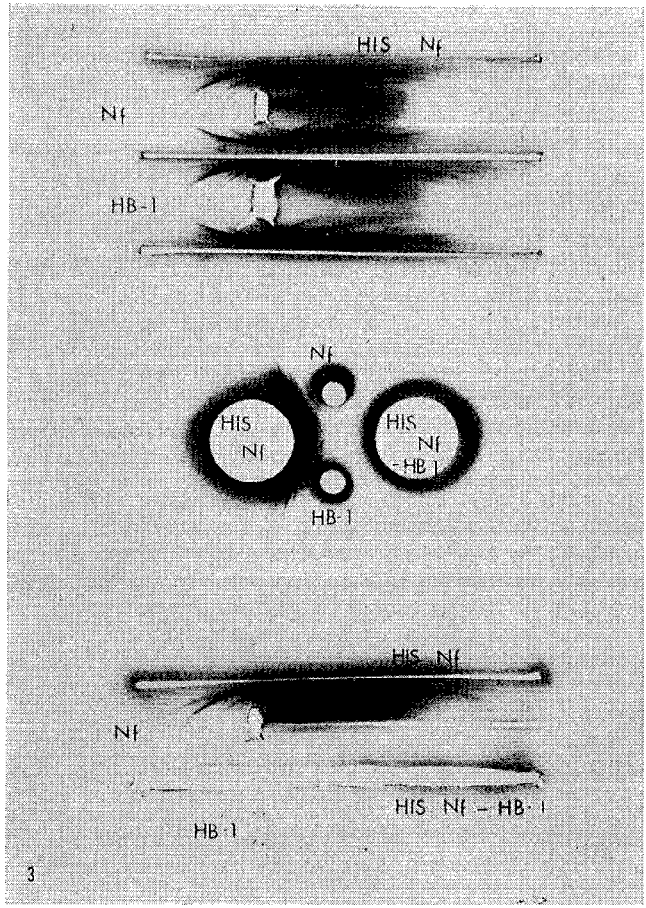


Figure 3
Immunoelectrophoretic pattern of an HIS anti-*N. fowleri* (Morgan) opposed to strain HB-1 showing an isologous antigenic structure.

The identical immunostructural nature of *N. fowleri* Morgan, *N. aerobia* and *N. invades* HB-1, supported by their identical morphological, biological and serological characters, confirms the priority of *N. fowleri* (Carter, 1970) of which *N. aerobia* and *N. invades* are junior non valid synonyms.

2. *Environmental strains*

2.1. Strains isolated from water supplies.

The epidemiology of PAME in South Australia presents a confusing picture.

Three northern towns, separated from each other by about 80 kilometers, have recorded cases during the summermonths for a period of at least seven years. There was an increasing incidence of cases going north with two at the most southern town, three in the next and eight in the northern-most town. No clear association with swimming could be established in any of the towns and, in some cases, no history of swimming at all could be elicited. These unusual circumstances prompted an intensive search for the causative organism in the environment, since the disease could clearly not be controlled as in other countries, by closing a specific pool.

2.1.1. Eight strains of pathogenic *Naegleria* were isolated from the reticulated water supply in South Australia. These have been confirmed as *N. fowleri* by the agglutination test and mouse pathogenicity test. All strains were agglutinated to a titre of 1 : 1024, which is the titre of the homologous reaction.

Moreover some of these strains have been examined by IEA.

2.1.2. Strain PA90 was isolated from a sample of main's tap water in Port Augusta in a district near to a proven case of PAME.

Strain PA117 was isolated from a tap in a vacant house in Port Augusta. Hydrosoluble extracts of these strains, opposed to an HIS anti-*N. fowleri* before and after absorption by the heterologous antigen, showed a complete isologous structure with *N. fowleri* of human origin (figures 4 and 5).

2.1.3. One of these eight sero-positive and mouse pathogenic strains called PA14 was obtained from a tap in the house of a fatal case of PAME (Port Augusta), where the child had been totally immersing himself in bath water during a period of very hot weather. The water supply is unfiltered river water, which was not adequately chlorinated. There was no history of swimming in a common swimming pool.

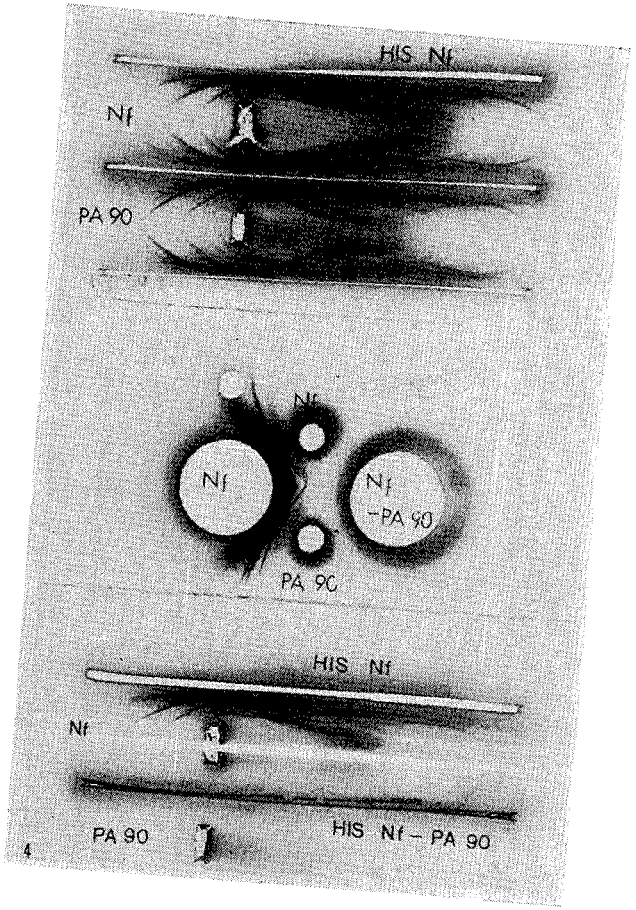
2.1.4. Recently strain PP397 (Jamieson, 1973) was isolated from flood drainage water. Torrential rain had followed a heat wave in an area where cases of PAME have occurred. Two of six mice inoculated intranasally with this uncloned strain died of PAME. The pathology was unusual in that there was no haemorrhagic necrosis of the olfactory lobes and very few amoebae could be seen in suspension of brain tissue. The amoebae were able to grow on agar plates, but not in axenic culture. A higher inoculum of this population produces disease in all mice inoculated but no evidence of microscopic involvement of the olfactory lobes and little disruption of tissue was seen in histological sections.

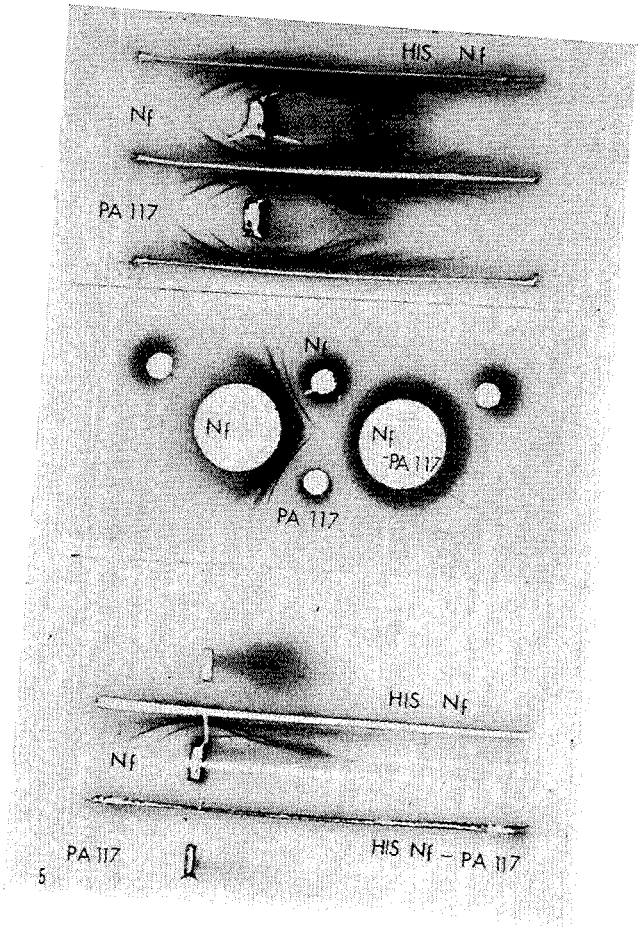
2.1.5. At the same time in New Zealand, strain NZ17 (Jamieson, 1973) was isolated from a thermal spring. Only one of the six mice inoculated was killed and amoebae were seen and cultured. This uncloned strain did not show the same serological behaviour as the human pathogens and has failed to kill mice since, even when large amounts of amoebae were inoculated.

Strains PP397 and NZ17 are still examinated.

2.2. *Strain isolated from dry soil*

2.2.1. In June 1972, a *Naegleria* strain PAa (Anderson *et al.*, 1973) was isolated from dry soil in an arid region near one of the towns where cases





Figures 4, 5

Immunoelectrophoretic pattern of an HIS anti-*N. fowleri* (Morgan) opposed to strains PA [illegible] and PA 117 (figure 5) showing complete [illegible]

of PAME have occurred. This uncloned strain produced fatal PAME in only one mouse out of six inoculated with 65,000 trophozoïtes. Subsequently all mice inoculated with similar doses died. Invasion of amoebae was confirmed by culture on agar plates and by histological sections. Serological analysis was not possible because the strain was unable to grow axenically. Between late August and December, all attempts to produce disease in mice were unsuccessful. In December 1972, axenic growth was achieved and the strain was found to be serologically negative. The strain was inoculated into six mice and all mice died of PAME. The strain isolated from these mouse brains, before, as well as after cloning, was found to be serologically positive.

2.2.2. A hydrosoluble extract made of a cloned culture of this strain was opposed to an HIS anti-*N. fowleri*, before and after absorption, but it did not show a completely isologous structure. A second batch of antigen was prepared because poor quality was suspected in the first batch. Results obtained with this new batch of antigen confirmed our first observation. An HIS anti-*N. fowleri* absorbed with the PAa antigen still revealed at least one antigenic component in homologous reaction, while in heterologous reaction no more components were observed (figure 6). It seems

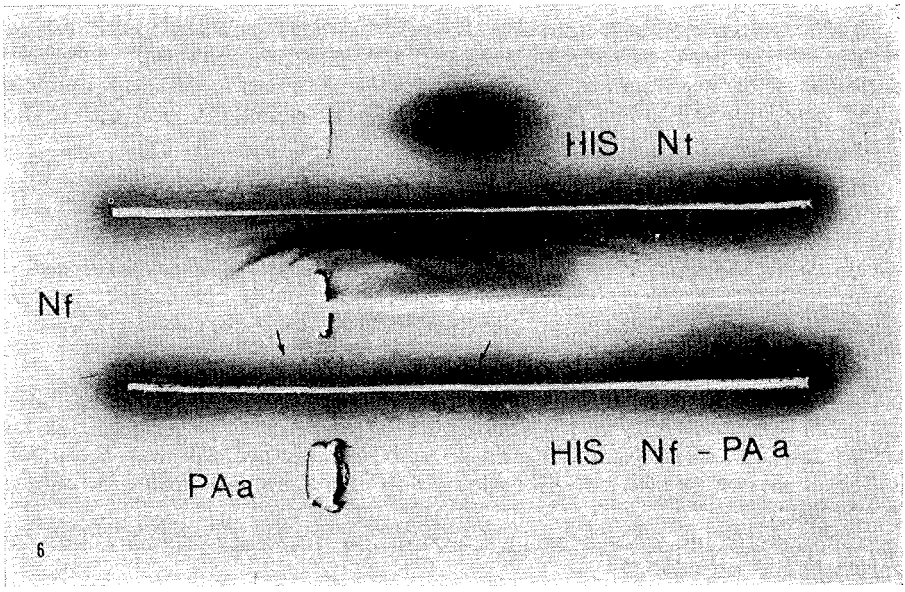


Figure 6

Immunoelectrophoretic pattern of an HIS anti-*N. fowleri* before and after absorption with strain PAa showing at least one antigenic component (arrow pointed) in homologous reaction after absorption of the *N. fowleri* antiserum.

from this that *N. fowleri* differs from *Naegleria* sp. PAa by at least one component. This result remains to be confirmed by a HIS anti-*Naegleria* sp. PAa and by an antigenic extract of another strain from the same origin.

Discussion

The reproducibility and the accuracy of the immunoelectrophoretic analysis credit this method with the privilege for identification and the comparison of amoebae belonging to the genus *Naegleria.*

1. Immunoelectrophoretic analysis confirms and stresses the existence of a genetic expression specific of the species designated as the human pathogen, *N. fowleri* (Carter, 1970).

The identical immunostructural nature of six human strains, originating from Czechoslovakia, U. S. A., New Zealand, Australia and Belgium enables us to confirm the geographical antigenic homogeneity of this species.

2. *N. fowleri* ordinarily has a free-living existence. In South Australia several strains of *N. fowleri* were isolated from water, sometimes in association with cases of primary amoebic meningoencephalitis, and one isolate was recovered from soil.

Not only in Australia have mouse pathogenic strains of *Naegleria* been isolated from the environment but also in other parts of the world. Singh and Das (1972) reported the isolation of pathogenic *Naegleria* from sewage sludge in Lucknow, India. Gordeeva (1973) also isolated two strains of mouse pathogenic *Naegleria* from the « cake » of sludge from sewage farms in the Moscow and Gorky districts.

Two strains isolated from tap water in Australia were identified by means of IEA as *N. fowleri* and showed a completely isologous antigenic structure with the human pathogens. However some confusing aspects were found among some strains on the immunological as well as on the biological level.

3. An antigenic difference concerning at least one component was observed between *N. fowleri* from human origin and the strain PAa recovered from dry soil. This difference remains to be confirmed.

Nevertheless, this cloned strain shows apparently the same morphological, biological and serological characters as the human *N. fowleri* strains, so it may be encountered in man. This strain appears to be a variant of the species *N. fowleri.*

4. Strain PP 397, which is pathogenic for mice and morphologically identical with *N. fowleri* is still unable to be grown axenically, while as far as we know all other pathogens do so without difficulty. Another confusing aspect is shown by strain NZ17 which killed just one mouse after intranasal inoculation.

Amoebae which were seen in the brain are growing axenically, but are serologically negative.

Moreover, they have failed to kill mice since, even with large doses of amoebae. In these uncluned populations of *Naegleria* amoebae it is possible that numbers of different species of *Naegleria* are present.

The possibility of competition among members of this heterogeneous population is considered, under the influence of external factors, resulting in the predominance of certain members of this population.

Considering the mouse brain as a biological filter, the possibility of eliminating non pathogenic members of a population could be taken into consideration.

In conclusion, a genetic homogeneity among human strains of *N. fowleri* is encountered, while amongst the pathogenic free-living strains a certain heterogeneity is observed and some strains may be considered as variants of the pathogenic human species. However, more information is required about these strains before making definite conclusions.

Acknowledgements — *Our thanks are due to Dr R. F. Carter (Adelaide) for histopathological comments on mouse brain sections and to Mr R. Moret (Antwerp) for his skilful assistance. This work was performed in relationship with the « Service d'Immunologie et de biologie parasitaire » (Prof. A. Capron), Faculté de Médecine de Lille (France).*

Samenvatting — **Epidemiologische en immunoelectrophoretische waarnemingen nopens *N. fowleri*, afgezonderd bij de mens en uit de omgeving.**

Primaire amoeben meningo-encephalitis (PAME) in Zuid-Australië schept een verward beeld in vergelijking met de gevallen in andere delen van de wereld, vermits hier geen verband met zwemmen werd vastgesteld. Nochtans, bij een *N. fowleri* stam afgezonderd bij de mens en twee stammen afgezonderd uit het drinkwater, werd een antigenische overeenstemming vastgesteld met stammen afgezonderd bij PAME afkomstig uit andere kontinenten.

Bovendien werden in Zuid-Australië stammen afgezonderd die als varianten van de pathogene *N. fowleri* zouden kunnen beschouwd worden.

De immunoelectrophoretische analyse word beschouwd als een veelbelovende techniek in de identificatie van *N. fowleri* stammen.

Résumé — **Etude épidémiologique et immunoélectrophorétique de *N. fowleri* d'origine humaine et libre.**

La méningo-encéphalite amibienne primitive en Australie méridionale présente un aspect particulier comparé aux cas dans d'autres pays, puisque aucun antécédent de natation n'a été observé. Néanmoins, une souche de *N. fowleri* isolée à partir d'un cas de MEAP et deux souches isolées de l'eau de réseau ont montré une identité antigénique complète avec d'autres souches d'origine humaine dans d'autres continents.

D'autres souches isolées en Australie méridionale, peuvent probablement être considérées comme des variantes de l'espèce *N. fowleri*.

L'analyse immunoélectrophorétique est considérée comme une technique importante dans l'identification et la comparaison de souches de *N. fowleri*.

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DISCUSSION

T. M. Preston : Mr Willaert have you had an opportunity to test the similarity or variance among the insoluble proteins, by producing antibodies against these ?

E. Willaert : No, we only analyzed the hydrosoluble proteins of *Naegleria* species.