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Office

WARNING

AMOEBIC MENINGITIS

In all thermal pools

KEEP YOUR HEAD ABOVE WATER

**to avoid the possibility of
developing the serious illness
called AMOEBIC MENINGITIS.**

**This disease can be caught in
thermal pools if water enters the
nose, while swimming or diving.**

PATHOGENIC FREE-LIVING AMEBAE-
OCCURRENCE IN NEW ZEALAND THERMAL REGIONS
TOGETHER WITH INVESTIGATIONS INTO THEIR
DISINFECTION, IMMUNITY AND VIRULENCE

A thesis presented in partial fulfilment of
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Raymond Thomas Michael Cursons
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ABSTRACT

Pathogenic free-living amebae (PFLA), of which Naegleria fowleri was the predominant pathogenic species, were isolated from 6 out of 10 pools sampled from the Hamilton, Rotorua and Gisborne Health Districts. The majority of these PFLA contaminated-pools occurred in the Matamata-Taupo region, and this localized geographical distribution appeared to be influenced, in part, by the particular physical and chemical properties of the pool. 'High-risk' pools, which exhibited a high incidence of isolations of PFLA, were shown to be natural pools, i.e. soil enclosures, as opposed to concrete constructed pools. PFLA were also isolated from the soil, and it was thought that soil acted as a reservoir of PFLA. No seasonal distribution in the occurrence of PFLA in thermal pools was noticed.

A comparative study on the disinfecting potential of chlorine, chlorine dioxide, ozone and deciquam 222 for PFLA showed that all 4 disinfectants possessed amebicidal properties, but only at higher levels than those normally used for disinfecting bacteria. Of the 4 disinfectants, deciquam 222 proved to be the most effective amebicide, followed by chlorine, chlorine dioxide and ozone.

An immunological survey of normal human sera for the presence of antibodies to either pathogenic or non-pathogenic Naegleria and Acanthamoeba spp. established that human sera had a titre ranging between $1/5$ - $1/20$ for Naegleria spp. and between $1/5$ - $1/80$ for Acanthamoebae. No discrimination in titres was observed between blood groups or sexes and fluorescein-labelled class-specific immunoglobulins showed that these antibodies belonged mainly to both the Ig M and Ig G classes. It was also shown that fresh adult human sera, as opposed to cord or specific hyperimmune-rabbit sera, contained a heat-labile neutralizing factor which inhibited the formation of cytopathic effects (CPE) in Vero cell

culture by Acanthamoeba culbertsoni, but not by N. fowleri. Homologous, as well as heterologous antigens of Naegleria spp. were however, shown to cross-react with both the in vitro, macrophage inhibition factor assay, and in vivo, delayed hypersensitivity, correlates of cell-mediated immunity.

Finally, this study also demonstrated that both pathogenic and non-pathogenic species of Naegleria and Acanthamoeba secreted both an extracellular phospholipase 2 and lysophospholipase into their axenic cultures. The relative production of phospholipase 2 correlated with the formation of CPE in Vero cell culture by either amebae, or by cell-free filtrates from axenic cultures of amebae. The relative level of production of this enzyme appeared to influence the virulence and hence pathogenic-potential of these micro-organisms.

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PREFACE

Since the first reported cases in New Zealand in 1968 of primary amebic meningo-encephalitis (PAM) (Mandal et al., 1970) the small free-living amebae of the genera *Acanthamoeba* and *Naegleria* have been of great interest to the New Zealand Department of Health. Locally it is known as 'Hot-Pool Meningitis' and there have been 7 confirmed cases of the disease all due to *Naegleria fowleri* and all contracted after swimming in thermal pools in the central North Island (Cursons et al., 1978b). Subsequently, these pathogenic free-living amebae (PFLA) (Adam et al., 1971) have been shown to be responsible for a number of diseases ranging from chronic illnesses such as respiratory infections (Martinez et al., 1975) and humidifier fever (M.R.C. Symposium, 1977) to blindness (Visvesvara & Jones, 1975).

The apparent ubiquity and ease of isolation of these amebae from the environment stimulated a program (initiated by the New Zealand Department of Health and run by the National Health Institute) of isolating amebae from specific thermal pools. Later this program was extended under contract to Massey University to cover 10 thermal pools in 3 different Health Districts, with the aim of sampling the pools for the presence or absence of PFLA and trying to correlate their presence or absence with such parameters as the chemical composition of the waters, pH, temperature, and numbers of bacteria. In addition, comparative disinfection tests were carried out using a variety of compounds to observe their respective amebicidal properties. Because of the repeated isolations of PFLA from some New Zealand thermal pools, an immunological study was also undertaken. It was hoped to explain the enigma of the human population's apparent immunity to these amebae, despite their pathogenicity and distribution. Finally, the virulence of these amebae in relation to their pathogenicity was also studied.

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