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Identification and Drug Sensitivities of *Acanthamoeba* Species Causing Keratitis

A Thesis Presented in Partial Fulfillment of the
Requirements for the Degree of
Master of Science in Microbiology
at
Massey University,
Palmerston North,
New Zealand.

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1997

Abstract:

Acanthamoebic keratitis is a distinct, vision-threatening ophthalmological condition, the incidence of which is increasing with increased usage of contact lenses. Diagnosis can be difficult and chemotherapeutic treatment is prolonged and often ineffective.

It is therefore desirable to discover a quick and accurate means of diagnosing acanthamoebic keratitis, and to gain knowledge regarding which chemotherapeutic agents are most effective against acanthamoebic keratitis.

The first goal of this thesis was to examine ten DNA extraction procedures and determine their effectiveness in extracting DNA from *Acanthamoeba* cells.

Of these ten methods, four (2, 7, 8, 10) could be performed in less than one day and showed consistent results in PCR reactions.

The second goal of this thesis involved the application of arbitrarily primed polymerase chain reaction (AP-PCR) in an attempt to type and group strains of *Acanthamoeba* species.

Examination of 16 isolates with primers APO1, APO2, AM1, AM2, P1 and P2, showed each of the banding patterns, resulting from AP-PCR analysis, were unique to the isolate tested. Further, there were few bands which occurred in more than one isolate, with insufficient similarities to form groupings of isolates.

Two chemotherapeutic agents were selected for a preliminary study into drug sensitivities in *Acanthamoeba* species and strains.

The first of these was Baquacil (20% polyhexamethylene biguanide (PHMB)), and the second was Brolene (0.1% propamidine isethionate).

Within 48 hours 97% of all isolates tested reached zero viability at a concentration of 0.05% PHMB, and 100% of isolates tested reached zero viability at 0.1% PHMB. The results of this study would suggest that the concentration of PHMB be at least 0.05% when used to treat acanthamoebic keratitis.

Within 48 hours of exposure to 0.1% propamidine isethionate (Brolene), only 30% of all isolates tested reached zero viability. However, 60% of isolates tested showed at least 80% reduction in viability within 48 hours of exposure to 0.1% propamidine isethionate.

The ultimate goal of this thesis was to form groups of isolates using PCR and drug sensitivities and to discover any correlation between these groups. The results of AP-PCR analysis however suggests a high genetic heterogeneity within the *Acanthamoeba* genus, thus preventing any correlation with drug sensitivity tests.

Acknowledgements:

This thesis was not a solo effort, a lot of people helped in many different ways, so I'd just like to take this opportunity to say a BIG thanks to all of them.

The Lab Rats:

- The Department of Microbiology and Genetics, Massey University, for providing the opportunity and facilities for this study.
- My Supervisors, **Dr Ray Cursons** for all his help, positive encouraging manner and for arranging funding for this project, and **Professor Tim Brown** for taking a chance on me and for providing motivation whenever I was almost ready to give up.
- The staff within the Protozoa Research Unit, especially Kirsty Farrant and Trish McLenachan for teaching me all about PCR and other useful stuff.
- All the staff and students within the Department who have lent a hand or just made me laugh - it's been a fun few years!
- ICI Chemicals for kindly donating the Baquacil used in these studies.

The 33'ers and The Hot Shots

I'm sorry, there's just not enough room to mention you all but thanks to each of you for listening to my ravings, making me laugh - lots, and for always understanding why I didn't go out, write or shoot, as often as we all would have liked. I don't know what I would have done without you!

I especially want to say a huge thanks to Debs and Manda for being the bestest friends I could ever wish for.

My Family

Mum, Dad and Helen. I think I must have the best family ever! Thanks for all your love and support, for always standing by me and believing in me - even, or especially, when I didn't believe in myself.

This Masterate belongs as much to you as it does to me. I love you.

And finally, I will acknowledge my own efforts. I didn't give up, when that seemed the only course, and now that I'm finished this part of my life I find myself secure in the knowledge that, whatever comes next, I can do it.

More than just science, this degree has taught me many skills I can use in all facets of my life. I guess the SGHS motto makes sense after all - Non Scholae et Vitae Discimus, Not for School but for Life we are Learning.

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CHAPTER ONE: Introduction

1.1 PATHOGENIC FREE-LIVING AMOEBAE

In the late nineteenth century amoebae were implicated in cases of dysentery and of organ and tissue abscesses (Wilhelmus, 1991). This was the first time amoebae were identified in human disease. These amoebae were parasitic, that is, needing a host to survive e.g. *Entamoeba histolytica*. Since then normally free-living (without need of a host) amoebae have also been recognised as opportunistic agents of human disease.

Of all free-living amoebae only those of the genera *Naegleria*, *Acanthamoeba* and *Balamuthia* have been shown to cause disease in humans and other animals. (Visvesvara *et al*, 1993)

These amoebae are able to live free in the environment, and as endoparasites. Normally they do not cause disease but live as phagotrophs, found in large numbers in soil, water and air, feeding on bacteria. (Anon., 1988 & John, 1993) As opportunists they may produce serious infection of the central nervous system (CNS) or the eye. (John, 1993)

Naegleria fowleri, *Balamuthia mandrillaris* and several species of *Acanthamoeba* are able to cause an often fatal infection involving the CNS, amoebic meningoencephalitis. (Visvesvara *et al*, 1993)

Additionally, some species of *Acanthamoeba* are able to produce a chronic, vision-threatening infection of the cornea, known as acanthamoebic keratitis. (John, 1993)

1.2 HISTORY OF ACANTHAMOEBA (John, 1993 & Wilhelmus, 1991)

- | | |
|---------|---|
| 1930 | Castellani mentioned the presence of a special amoeba in cultures of <i>Cryptococcus pararoseus</i> and this was classified in the subgroup <i>Hartmannella-Acanthamoeba</i> .(John, 1993) |
| 1957-58 | Two scientists, Culbertson and Jahnes, detected <i>Acanthamoeba</i> species as contaminants of tissue cultures causing cytopathic effects in Monkey kidney cell cultures. (John, 1993 & Wilhelmus, 1991) |
| 1958-59 | It was proposed that these amoebae have pathogenic potential. This was demonstrated when <i>A. culbertsoni</i> was inoculated intracerebrally and intravenously into monkeys and mice causing meningoencephalitis. (John, 1993) |
| 1971-73 | The first clearly identified <i>Acanthamoeba</i> infections of the CNS in humans occurred. Prior to this date these amoebae were occasionally referred to as <i>Hartmanella</i> species, although it is now commonly accepted there are no (known) pathogenic <i>Hartmannella</i> species. (John, 1993) |
| 1974 | The first two cases of acanthamoebic keratitis were reported from Great Britain. (John, 1993) |

- 1975 Amoebae cultured from both (above) British patients were identified as *Acanthamoeba* species. (John, 1993)
The first United States cases of acanthamoebic keratitis were reported. (John, 1993)
- 1980 The term granulomatous amoebic encephalitis (GAE) was proposed to describe the CNS infection caused by *Acanthamoeba* species. (John, 1993)
- 1983 *Acanthamoeba* species were isolated and cultured from human tissue samples for the first time. (John, 1993)
- 1985-95 Dramatic increase in numbers of acanthamoebic keratitis cases reported. This increase has been linked to the wearing of contact lenses, especially soft contact lenses. (John, 1993 & Wilhelmus, 1991)
- 1996 To date there have been six cases of acanthamoebic keratitis reported in New Zealand. (Cursons, *pers comm.*)

1.3 BIOLOGY

1.3.1 Morphology:

Acanthamoeba is found in two morphologically distinct forms; an environmentally resistant cyst and a feeding trophozoite or amoeba. It is the amoeba which is infective but both forms have been found in human tissue. (Anon, 1988 & John, 1993)

The trophozoites are uninucleate, with characteristic fine cytoplasmic projections (acanthopodia) and the cysts are also uninucleate having a resistant stellate double wall. Both forms are resistant to most antimicrobial agents. (Anon, 1988) The nuclei of *Acanthamoeba* are characterised by having a large central nucleolus, or karyosome, and a nuclear membrane without chromatin granules. (John, 1993)

1.3.1.a. Trophozoite:

A distinguishing feature of the *Acanthamoeba* genera is the presence of acanthopodia, tapering spike-like pseudopodia. *Acanthamoeba* moves slowly on a broad front without direction and trophozoites average about 24-56µm in length. Nuclear division is metamitotic, where the nucleolus and the nuclear membrane disintegrate during early karyokinesis. (John, 1993)

The cytoplasm consists of an amorphous hyaloplasm in the periphery emerging gradually with the granular endoplasm and its array of organella, mitochondria, ribosomes, phagocytic and pinocytic vacuoles, the pulsative complex, the centrosphere (essential for mitosis) and the nucleus. The numerous mitochondria usually have club-like or ovoid shape. (Rondanelli and Scaglia, 1987)

1.3.1.b. Cyst:

The cystic stage is preceded by several morphologic and functional changes among the cytoplasmic organelle contained in the trophozoite. (Rondanelli and Scaglia, 1987)

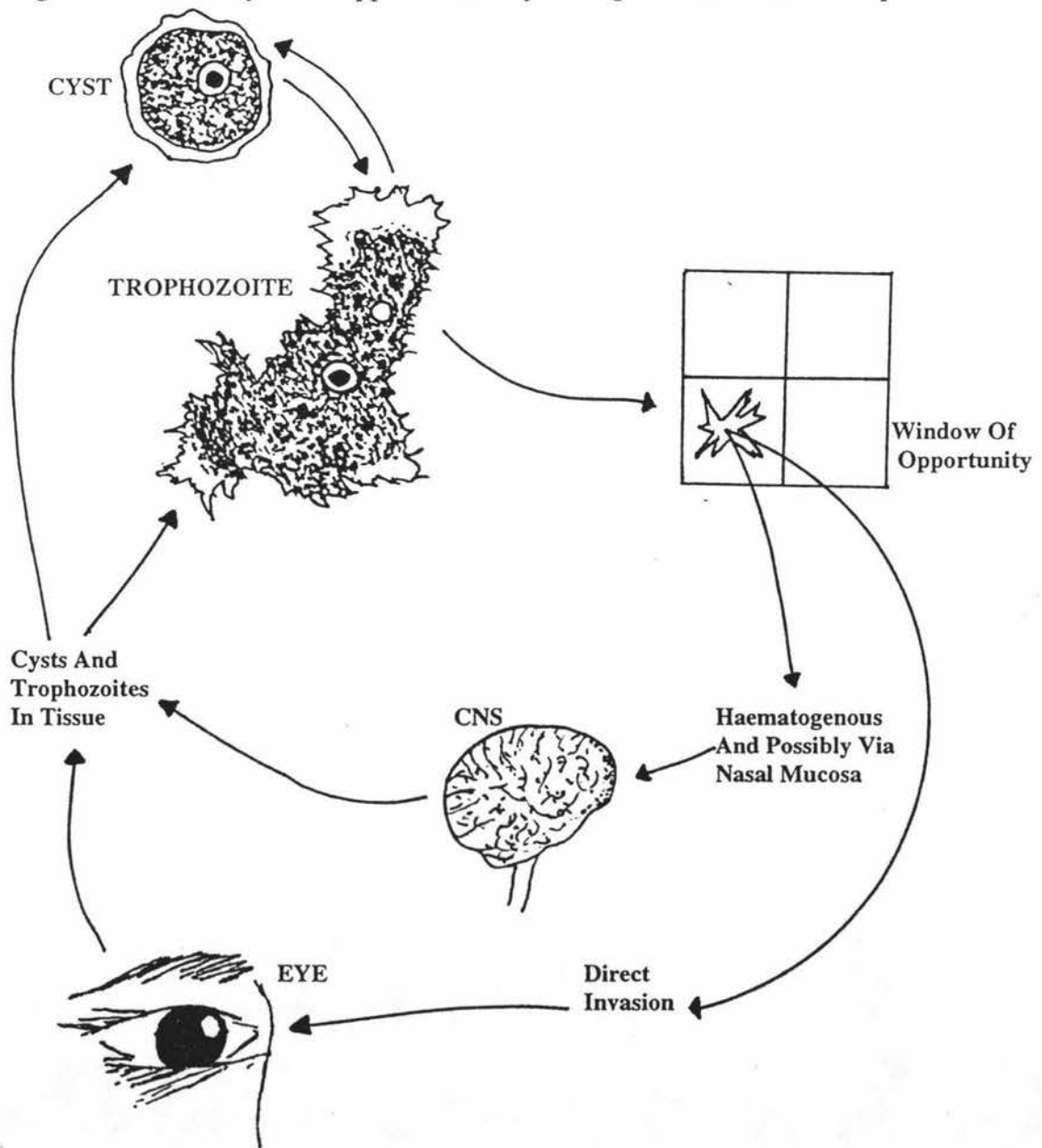
Considerable variation in cyst morphology occurs among the different species and this has been used in the naming of species. (John, 1993)

Cysts are double walled and, therefore, quite resistant in the environment. The cyst wall is made up of an outer wrinkled, or rippled, ectocyst and an inner endocyst. (John, 1993) The two membranes appear separate along most of the cell perimeter and join only in areas corresponding to the tips of the polygonal limbs, where they form pores. A metacystic trophozoite is believed to break through these to leave the cyst. (Rondanelli and Scaglia, 1987)

The karyosome in the cyst is smaller than that of the trophozoite and the granular cytoplasm contains many empty vacuoles and liquid droplets which, at times, line the whole inner surface of the cellular membrane. (Rondanelli and Scaglia, 1987)

1.3.2 Life Cycle:

Figure 1.3.2: Life Cycle of Opportunistically Pathogenic *Acanthamoeba* Species.



Invasion of the CNS appears to be by way of the circulation, with amoebae originating from a primary focus elsewhere in the body, possibly the respiratory tract, or ulcers of the skin or mucosa, or other wounds. (Ma *et al*, 1990 & John, 1993)

GAE tends to occur in persons who are debilitated, chronically ill, or immunocompromised. In contrast acanthamoebic keratitis usually occurs in healthy individuals, and infection is by direct invasion of the cornea through trauma to the eye or the wearing of contaminated contact lenses. (John, 1993)

1.4 CLASSIFICATION

The genus *Acanthamoeba* was established in 1931. Since then *Acanthamoeba* species have been variously referred to as *Acanthamoeba*, *Hartmannella*, and *Mayorella*.

In 1930 Castellani discovered an amoeba in a culture of the fungus *Cryptococcus pararoseus*. Douglas placed this amoeba in the genus *Hartmanella* that same year.

Later, the genus *Hartmanella* was considered an artificial assemblage of unrelated amoebae and was subdivided into three genera, one of which was *Acanthamoeba*.

These amoebae are characterised by the appearance of pointed spindles at mitosis and have double walled cysts with ostioles and an irregular outer layer.

Between the years 1952 and 1979 there were continuing debates regarding the validity of the genus *Acanthamoeba*. These culminated in the acceptance of 17 species in the genus *Acanthamoeba*. Seven of these have been shown to be pathogenic to humans with a further three species pathogenic to mice. The currently accepted position of *Acanthamoeba* in the taxonomic scheme of the Society of Protozoologists is shown in Table 1.4.1 (Visvesvara, 1991).

An additional classification system that is generally accepted is that of Pussard and Pons (1977) which uses cyst morphology to divide the genus into three groups.

Strains isolated from human infection fall into groups II and III with the majority of corneal isolates falling into group II.

Although the genus *Acanthamoeba* is easily recognised because of its distinctive acanthopodia and cyst structure, species identification is rather difficult.

Traditional taxonomic criteria have been based on cyst morphology, nuclear divisions, temperature tolerance, and pathogenicity. The more recent approaches to *Acanthamoeba* classification have used isoenzyme analysis, restriction enzyme analysis of mitochondrial DNA and analysis of random fragment length polymorphism of total cellular DNA. (Cursons, 1978 & Ma *et al*, 1990). However, the results of these analyses often have not correlated with species identification based on the more traditional criteria.

In the end, the best classification scheme will be one that relies on many different characteristics, including morphological, physiological and biochemical ones. (John, 1993)

Table 1.4.1: Currently Accepted Taxonomy (Visvesvara, 1991 & Ma *et al*, 1990)

Kingdom:	Protista
Subkingdom:	Protozoa
Phylum:	Sarcomastigophora
Subphylum:	Sarcodina
Superclass:	Rhizopodia (locomotion by lobopodia, filopodia, rectilopodia, or protoplasmic flow without production of discrete pseudopodia)
Class:	Lobosea
Subclass:	Gymnamoebia
Order:	Amoebida (typically uninucleate; mitochondria present; no flagellate stage)
Suborder:	Acanthopodina (more or less finely tipped, sometimes filiform, often furcate hyaline subpseudopodia produced from a broad hyaline lobe not regularly discoid; cyst usually formed; nuclear division mesomitotic or metemitotic).
Family:	Acanthamoebidae
Genus:	<i>Acanthamoeba</i>
Species:	Isolated from human infections at indicated site(s):
	<i>A. astronyxis</i> (CNS)
	<i>A. castellani</i> (CNS, eye)
	<i>A. culbertsoni</i> (CNS, eye)
	<i>A. hatchetti</i> (eye)
	<i>A. palestinensis</i> (CNS)
	<i>A. polyphaga</i> (CNS, eye)
	<i>A. rysodes</i> (CNS, eye)
	<i>A. lugdunensis</i> (eye)

Not isolated from human infections:

A. comandoni

A. griffini

*A. lenticulata**

*A. royreba**

A. tubiashi

A. enchinulata

A. divionensis

A. triangularis

A. mauritaniensis

* shown to be pathogenic for mice

1.5 EPIDEMIOLOGY

1.5.1 Occurrence/distribution of *Acanthamoeba* species:

Acanthamoebae appear to be truly ubiquitous organisms with isolations recorded from: freshwater, bottled mineral water, frozen swimming water, distribution (i.e. tap) water, chlorinated swimming pools, physiotherapy pools and medicinal pools, hot tubs, brackish and sea water as well as ocean sediments, soil, compost, sewage, mushrooms and vegetables, dust in air, cooling towers of electric and nuclear power plants, heating, ventilating and air conditioning units, bacterial, fungal and mammalian cell cultures, fish, reptiles, birds, mammals (intestines, cerebral tissue, lung tissue, skin wounds, corneas). In humans they have been found in the nose and throats of people with respiratory illness as well as from healthy persons, and in bronchial secretions, ear discharge, stool samples of patients with diarrhoea, intrauterine contraceptive devices, dialysis units, gastrointestinal washings, gastric-lavage tubing, dental units, contact lenses and contact lens solutions. (De Jonckheere *et al*, 1991 & Visvesvara *et al*, 1990)

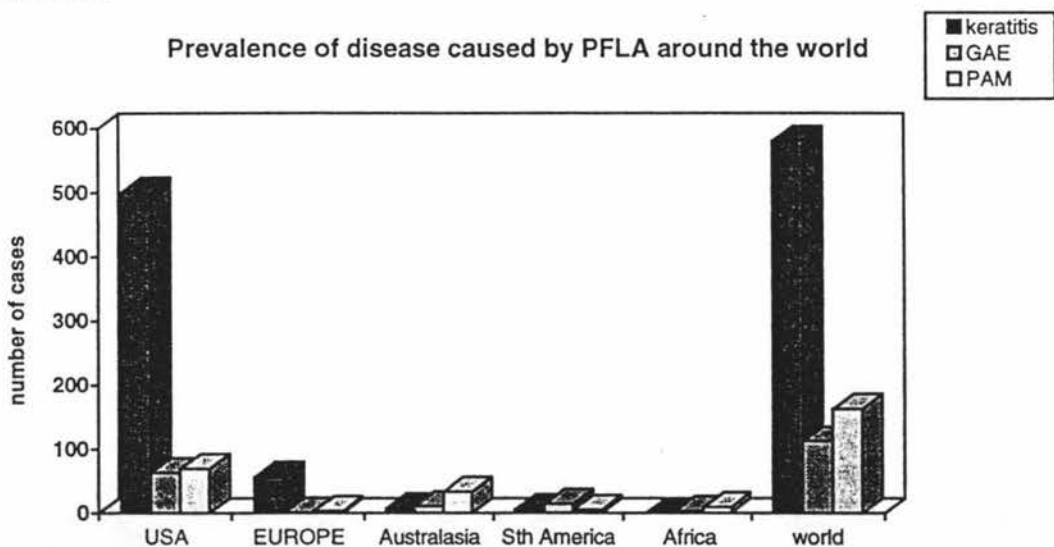
This apparently ubiquitous distribution is no doubt due to the possession of resistant cysts enabling these pathogenic free-living amoebae to withstand unfavourable conditions and to take advantage of the intermittent occurrence of favourable conditions. (Cursons, 1978)

The distribution of pathogenic species to non-pathogenic ones is still unknown. In general pathogenic species are found in conditions where temperatures are above ambient, and closer to body temperature. (Cursons, 1978)

1.5.2 Prevalence of disease caused by pathogenic free-living amoebae

The following graph provides a comparison of the numbers of Primary Amoebic Meningitis, Granulomatous Amoebic Meningoencephalitis and acanthamoebic keratitis in several areas of the world (Visvesvara, *pers comm.*).

Figure 1.5.1



In most countries acanthamoebic keratitis is not a notifiable disease, therefore the true incidence is not known. A study in the USA revealed that where clinicians were familiar with acanthamoebic keratitis the numbers of cases reported were higher.

The following graph compares reported numbers of acanthamoebic keratitis with those of GAE and PAM in the USA (Visvesvara and Stehr-Green, 1990).

Figure 1.5.2

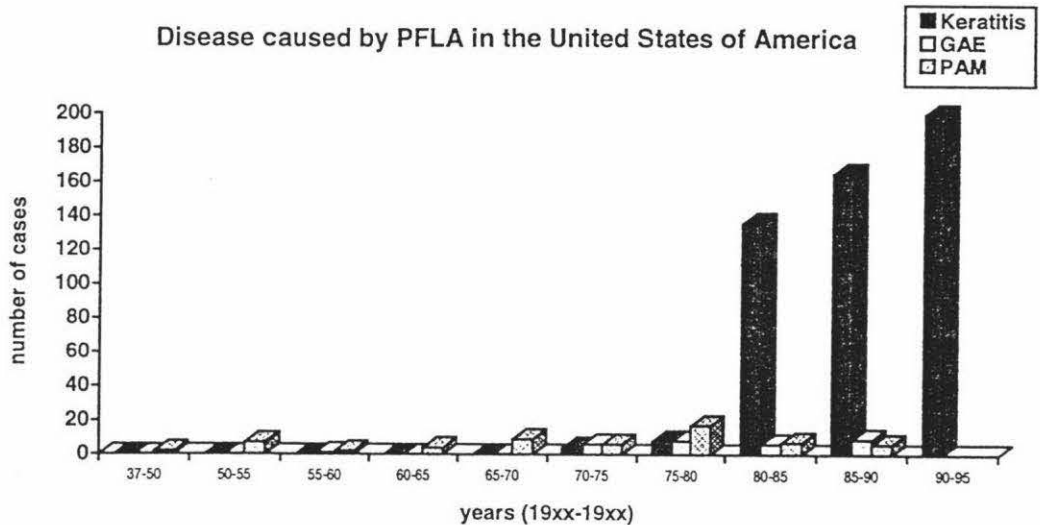


Figure 1.5.2 shows a dramatic increase in cases of acanthamoebic keratitis since 1980, while numbers of cases of PAM and GAE remain approximately static in occurrence. This increase correlates with increased usage of contact lenses. It has been suggested that contact lens use for purely cosmetic reasons is high in the United States of America. Therefore it would not be unreasonable to expect an increase in acanthamoebic keratitis in those areas of the world, shown in Figure 1.5.1, which currently have low contact lens usage, as contact lenses become more popular. It is also likely that although PAM and GAE appear to be of more importance in some areas, these diseases are not being reported at a higher frequency and acanthamoebic keratitis has the potential to overtake these statistics in a very short amount of time.

For example, in New Zealand the majority of reported PAM cases occurred in a short period of time in the early 1970's. Since that time perhaps 1 or 2 further cases have occurred. In comparison, 6 cases of acanthamoebic keratitis have been reported within the last 5 years (Cursons, *pers comm.*).

1.6 DISEASES PRODUCED

1.6.1 Granulomatous Amoebic Encephalitis (GAE):

GAE usually occurs in debilitated or chronically ill, or immunocompromised individuals, although some cases have been reported in otherwise healthy people.

This disease is not well defined with the course of infection being subacute or chronic and lasting from weeks to months, or maybe even years. Onset is insidious with a prolonged clinical course during which single or multiple space-occupying focal granulomatous lesions develop in the brain.

An altered mental state is a prominent feature in GAE, with headache, seizures and neck stiffness occurring in about half of the cases. Nausea and vomiting may be present in some cases.

Acanthamoeba infection probably occurs through the lower respiratory tract or through ulcers of the skin or mucosa. Invasion of the CNS occurs by haematogenous spread from the primary focus of infection. As the brain has no lymphatic channels the invasion must take place via the bloodstream. (John, 1993)

1.6.2 Acanthamoebic keratitis:

A chronic infection of the cornea caused by direct contact of the cornea with amoebae. This may occur through minor corneal trauma or exposure to contaminated water or contact lenses. (John, 1993)

Acanthamoebic keratitis usually develops over a period of weeks to months.

Characteristics of the disease include severe ocular pain, often out of proportion to the degree of inflammation, affected vision, and a paracentral corneal ring infiltrate.

If not correctly treated this infection can lead to loss of vision and, in extreme cases, removal of the infected eye may be necessary. This disease on occasion is mistakenly diagnosed as *Herpes simplex* keratitis, with the only major difference in symptoms being the severity of ocular pain occurring in acanthamoebic infections. (John, 1993).

This disease is further discussed later in this introduction.

1.7 IMMUNITY

Because of the frequency and ease of isolation of pathogenic free-living amoebae (PFLA) from the environment, many authors have been puzzled regarding the low incidence of infection by PFLA in the human population. This has led many authors to speculate upon the existence of probable host-related susceptibility factors although these remain undefined.

Antibodies to PFLA have been detected in normal human sera by indirect fluorescent-antibody testing. These have been to both pathogenic and non-pathogenic free-living amoebae (Cursons *et al.*, 1980 & John, 1993)

Predisposition to infection by contact lens wearers may be because,

1. the lens holds the amoebae in contact with the cornea,
2. the lens causes minor trauma, allowing the amoebae to enter through the epithelial layers,
3. the lens protects the amoebae from removal by blinking or tear secretions, and/or
4. levels of antibodies and complement are low in the eye.

1.8 ACANTHAMOEBIC KERATITIS

In 1973 Visvesvara reported the first case of acanthamoebic infection of the eye. (Asbell, 1993) Sporadic reports of ocular acanthamoebic infection followed; in 1982, not a single case of acanthamoebic keratitis was found in a 30-year review of 700 patients with corneal ulcers, all of whom had undergone laboratory evaluation. (Asbell, 1993) It was not until about 1985, after contact lens use, in particular soft and disposable (extended wear) contact lens use, had dramatically increased, that the

numbers of acanthamoebic keratitis cases being reported showed a marked increase and a direct association between contact lens wear and acanthamoebic keratitis was recognised. (Asbell, 1993) Between 1980 and 1985 there was a 59% increase in numbers of people wearing contact lenses in the United States of America. (John, 1993) Acanthamoebic keratitis is a distinct, vision-threatening ophthalmological condition, causing serious concern in many countries. It is more common in economically developed countries where the wearing of contact lenses, for cosmetic and sight corrective purposes, is both fashionable and affordable. (John, 1993)

Corneal trauma often precedes infection but this may not be a necessity. (Moore, 1990). Even minor trauma caused during the insertion, removal, or normal wearing of contact lenses can provide an avenue for entry of the micro-organisms. (Asbell, 1993)

Because the clinical signs of acanthamoebic keratitis resemble herpetic, fungal or bacterial keratitis, diagnosis and treatment may be erroneous. (Horne *et al*, 1994)

1.8.1 Diagnosis:

Common clinical signs of ocular acanthamoebic infection are breakdown of the corneal epithelium, the presence of satellite lesions, and iritis, sometimes with hypopyon. Often, after an initial period, the infection progresses slowly. Infection waxes and wanes, with corneal healing followed by recurrent epithelial deterioration. This can present a confusing picture to the clinician in practice. There may be elevated intraocular pressure and scleral inflammation. (Asbell, 1993)

The primary sign of infection that has penetrated deep into the cornea is the combination of a central or paracentral ring infiltrate and remarkably intense pain. (Asbell, 1993)

The initial method of diagnosis is to take corneal scrapings for culture on non-nutrient agar seeded with bacteria, and subsequent microscopic examination. It is often useful to examine contact lenses, storage cases and care solutions (where appropriate) as well as corneal tissue for the presence of acanthamoebic contamination. In some cases, biopsy may be necessary to obtain a firm diagnosis. (Horne *et al*, 1994)

It is important that specimens be examined by someone who is thoroughly familiar with the appearance of *Acanthamoebae* because these micro-organisms may be confused with degenerated epithelium or macrophages. (Anon., 1988)

Species identification may be determined using a variety of staining techniques including indirect immunofluorescent antibody, Giemsa, and calcofluor white. Identification in histopathological specimens may be achieved by employing the haematoxylin and eosin procedure or a more specialised staining method e.g., Heidenhain's haematoxylin, Gomori's chromium haematoxylin, periodic acid-Schiff, Bauer chromic acid-Schiff and silver methanamine. (John, 1993)

Herpes simplex keratitis is the most common misdiagnosis. The single most consistent clinical symptom is severe ocular pain, which is not characteristic of infection limited to the cornea and generally not present in *Herpes simplex* infection. Additional distinguishing features include a history of direct exposure to soil or water, wearing contact lenses, scleritis, and failure of cultures from the inflamed eye to reveal bacteria, fungi or viruses. (John, 1993)

1.8.2 Pathology:

The pathology of acanthamoebic keratitis is characterised by chronic progressive keratitis (John, 1993). In the early period of infection pseudodendritic figures in the

epithelium or just beneath the epithelium, in the anterior stroma, are typical pathological features. (John, 1993)

This period is thus characterised by limbitis, perineural infiltrates and superficial epithelial changes, with few ring infiltrates and little anterior uveitis. (Bacon *et al*, 1993) As the infection progresses into the active period pathology includes frank ulceration, ring infiltrates and anterior uveitis often with hypopyon. Sometimes endothelial plaques causing corneal oedema occur. Perineural infiltrates are less common in early disease. (Bacon *et al*, 1993) In advanced cases there may be marked stromal infiltrate and necrosis. The whitish inflammatory infiltrate, often appearing ring-shaped around the corneal ulcer, consists mainly of polymorphonuclear leukocytes and macrophages with a few lymphocytes. (John, 1993) Evolution of severe features of the disease include ring ulcers, abscesses, scleritis, glaucoma, cataracts and secondary microbial infection. (Bacon *et al*, 1993).

Although granulomatous inflammation has been described in acanthamoebic keratitis (John, 1993), in most of the reports, neutrophils, and not lymphocytes, are the predominant infiltrating cells. Corneal ulceration may progress to perforation (John, 1993). Acanthamoebic ocular infections have been described variously as conjunctivitis, iritis, scleritis, and uveitis. (John, 1993).

Acanthamoeba trophozoites and cysts occur within infected tissue. (John, 1993)

1.8.3 Treatment:

Initially most patients required surgical treatment, ranging from surgical debridement of the affected cornea to corneal transplantation or removal of the infected eyeball. (Asbell, 1993 & John, 1993)

The first successful chemotherapeutic treatment used a combination of dibromopropamide and propamide isethionate. Treatment was prolonged but curative. (Asbell, 1993)

Current data suggest that cure is possible if acanthamoebic keratitis is diagnosed early, and appropriate medical treatment is initiated quickly and administered for a prolonged period i.e., six to twelve months. (Asbell, 1993)

1.8.3.1 Pharmacology:

The following drugs with varying efficacy have been used to treat cases of acanthamoebic keratitis.

Topical propamide, topical miconazole, systemic ketoconazole, neosporin topical clotrimazole, oral itraconazole, neomycin., polyhexamethylene biguanide (PHMB), paromomycin, acriflavine, polymyxin B, colistin, crystal violet, methyl benzalkonium chloride, an experimental drug (R11/29) and brilliant green.

In some patients a combination of between three and twelve of these drugs were used, sometimes in combination with surgical debridement of lesions. (Asbell, 1993, John, 1993, Bacon *et al*, 1993, Horne *et al*, 1994)

The most efficacious amoebistatic agents at present are pentamidine isethionate, propamide isethionate, stilbamidine isethionate, clotrimazole and ketoconazole. Ethanol and methanol have also showed activity against amoebae (Tomlinson, 1991).

Studies suggest that inhibitors of polyamine synthesis, e.g. diminazene aceturate, pentamidine and methylglyoxal bis(guanylhydrazone)(mitoguanone) could be developed to aid in chemotherapy for acanthamoebic infections. (Shukla, 1991)

Magainins from the skin of a south African toad were shown to be inhibitory to trophozoite growth and, although not cysticidal, may be useful in the management of acanthamoebic keratitis. (Feldman *et al*, 1991)

Local corticosteroids may be detrimental to the host in the presence of acanthamoebic keratitis. These were commonly used in early cases where prognosis was likely keratoplasty in order to limit inflammation and increase successful surgery. (John *et al*, 1991b)

1.8.4 Risk factors:

Risk factors involved in contraction of acanthamoebic keratitis include wearing contact lenses, use of nonpreserved ophthalmologic products (e.g., home-made saline), exposure of eyes to nonsterile water sources (e.g., well, tap, sea and surface water), contamination of contact-lens storage cases and of storage and rinsing solutions, trauma to the eye, compromised host defence mechanisms and corneal surgery. (Horne *et al*, 1994)

Eighty percent of reported cases of acanthamoebic keratitis have occurred among contact lens wearers, 75% of whom wore daily-wear or extended-wear soft hydrogel lenses.

There is also evidence to suggest that a great many lens wearers remove lenses, usually because of dust, dirt or ocular irritation, and reinsert them later after a rinse in tap water or saline, without disinfection. (Asbell, 1993)

The risk factors associated with contact lens wear include:

Lens materials. All modern contact lenses contain a certain amount of water which serves as a medium for oxygen influx. In proportion to their water content, lenses absorb material, including potential pathogens, from cleaning solutions, lens cases, and patients hands. Thus, lens material itself can be a risk factor for acanthamoebic infection. (Asbell, 1993)

Lens Cleaning and Disinfection. Preservatives in solutions were major culprits in discomfort. This led practitioners to recommend the use of unpreserved, home-made, solutions. At this time it was not known that *Acanthamoeba* was ubiquitous in the environment, and particularly in tapwater. The marked increase in acanthamoebic infections during the 1980s certainly reflected, to a degree, widespread use of non-sterile, unpreserved solutions. This practice is now actively discouraged. (Asbell, 1993) Other sources of lens contamination result from the patient not following disinfection regimes properly or rinsing lenses in tap water to remove disinfecting solution or dust particles. (Asbell, 1993)

Lens Storage Cases. In studies involving 102 asymptomatic lens wearers and their lens cases it was discovered that about half of the lens cases contained significant amounts of bacteria and seven cases contained *Acanthamoeba*. In general *Acanthamoeba* do not occur alone and it is possible a synergistic or symbiotic relationship with other bacteria allows *Acanthamoeba* to grow to levels where there becomes a significant risk of pathogenic infection.

At the moment there is no obvious solution to this problem (Asbell, 1993), although one suggestion is that the contact lens case be replaced with the purchase of disinfection systems.

Extended Wear. A study in 1989 showed that soft-lens wearers who slept with lenses in were 10-15 times more likely to develop ulcerative keratitis than those who wore lenses only during waking hours. (Asbell, 1993)

Lens Degradation. Contact lenses, over a period of time, eventually develop deposits. This is unavoidable and are in fact part of a biofilm. This biofilm is produced in response to a foreign body (the lens) enabling the body to recognise the lens and not react adversely to them. However it is also a source of contamination, adding to the risk of infection. (Asbell, 1993)

1.9 SUMMARY AND GOALS

There are few free-living amoebae that are also opportunistic agents of human disease. One genera of amoebae that is capable of this is *Acanthamoeba*.

Some strains of *Acanthamoeba* are able to produce one or both of two distinct disease states in humans. The first of these is Granulomatous Amoebic Encephalitis (GAE), an infection of the central nervous system that can be compared to amoebic meningitis (or primary amoebic meningoencephalitis - PAM). PAM is caused by another pathogenic free-living amoeba, *Naegleria fowleri*.

The second disease state caused by *Acanthamoebae* is a chronic infection of the cornea known as acanthamoebic keratitis.

All diseases caused by pathogenic free-living amoebae occur infrequently, however the incidence of acanthamoebic keratitis is increasing. This increase has been linked with the increased usage of contact lenses, and in particular, soft contact lenses. Once diagnosed, chemotherapeutic treatment is prolonged and often ineffective. This can render necessary surgical means of controlling the disease. Surgical options include debridement of lesions, keratoplasty or, in extreme cases, removal of the infected eyeball.

It is therefore desirable to discover a quick and accurate means of diagnosing acanthamoebic keratitis, and to gain knowledge regarding which chemotherapeutic agents are most effective against acanthamoebic keratitis.

The goals of this thesis are:

1. To compare methods of extracting DNA from *Acanthamoeba* species in order to
2. Evaluate the polymerase chain reaction as a means of diagnosis of acanthamoebic keratitis and differentiation of *Acanthamoeba* species and strains.
3. To conduct preliminary *in vitro* drug sensitivity trials.
4. To determine whether any correlation exists between drug sensitivities and banding patterns revealed by PCR.