SUMMARY

From May 1996 to August 1998, a total of 3252 patients with keratitis reported at the Regional Institute of Ophthalmology, Government Ophthalmic Hospital, Egmore. Based on the clinical features, Acanthamoeba keratitis was suspected in 488 patients. Acanthamoeba was grown in 52 of them. This constitutes 1.6% of the total corneal ulcers. Out of 52 Acanthamoeba cases, 28(54%) were males and 24(46%) were females. Pure Acanthamoeba was isolated from 9(17.3%) cases, Acanthamoeba along with Bacteria were isolated from 17(10%) cases. Acanthamoeba and fungi together were isolated from 20(11.8%) cases. In 6 (11.6%) patients, Acanthamoeba was associated with Fungi and Bacteria.

From 1993-1998, samples were obtained from Post Graduate Institute of Ophthalmology and Aravind Eye Hospital, Madurai under a collaborative project. A total of 5097 patients with keratitis reported to the cornea clinic during the study. Acanthamoeba was isolated from 73(1.4%) cases. Out of the 73 positive cases, 44(60.3%) were males and 29(39.7%) were females. Acanthamoeba was isolated in pure cultures from 62(85%) patients. Association of Acanthamoeba with bacteria was found in 9(12.3%) cases and Acanthamoeba with fungi in 2(2.7%) cases. The number of mixed infections reported from Madurai was comparatively lesser than Chennai.

Slide culture was used for isolation from clinical specimen and plate culture for propagation of Acanthamoeba spp. Both of them proved to be efficient.
The slide culture was helpful in viewing the trophozoite directly and served as a transport cum growth medium for *Acanthamoeba* spp.

The growth pattern of *Acanthamoeba* on NNA in our study revealed that, it took a minimum of 5 days for the maturation of the cysts at 37°C. The excystation pattern of the three groups was different.

All the strains grew both at 25°C and 37°C. The cysts of *Acanthamoeba* failed to excyst at 4°C, 54(77%) strains grew at 42°C and 16(23%) failed to grow at 42°C.

Morphometrical analysis was carried on the mature cysts of 70 strains of *Acanthamoebae*. Based on the characteristics of the cyst 15(21.4%) were identified as Group I, 37(52.9%) as Group II and 18(25.7%) as Group III.

These three Groups of *Acanthamoeba* were selected at random to study the growth pattern on NNA. In group II and Group III the 3 cornered cysts showed similar pattern, however in Group I the number of 3 cornered cysts were comparatively very few. The growth pattern of the Group I Acanthamoeba was unique. Six cornered cysts were not seen in Group III, which is a characteristic feature of this Group.

In order to get pure viable cysts, *Acanthamoeba* cysts-*E. coli* mixture was treated with 1N HCl for 30 minutes. This treatment killed *E.coli* and did not affect the viability of *Acanthamoeba*.
Antiserum to three different groups of *Acanthamoeba* was raised in Rabbits. The highest titer was obtained from Group II followed by Group I and lowest in Group III. The peak titer was obtained on day 60 from all groups.

All the strains that were morphologically grouped were tested for their pathogenicity in mice. Out of the 70 strains tested only 34 (48.6%) killed the animal and the rest 36 (51.4%) were not pathogenic to mice.

In Rabbit model of keratitis, all the strains induced keratitis. The virulence pattern of the Group I *Acanthamoeba* isolates is as follows: out of the 6 strains tested, 2 (33.3%) strains were highly virulent, 3 (50%) were moderately virulent and 1 (16.7%) strain was less virulent.

Out of the 7 strains tested in Group II *Acanthamoeba* isolates, 1 (14.3%) strain was highly virulent, 2 (28.6%) were moderately virulent and 4 (57.1%) were less virulent. More number of less virulent strains was found in this Group.

Out of the 17 strains tested from Group III isolates, complete data were available for only 11 strains. Among these 11 strains 2 (18.2%) were highly virulent and 9 (81.8%) were moderately virulent. Less virulent strains were not prevalent among the Group III *Acanthamoeba*.

Both humoral and cell mediated immune responses were studied in experimental *Acanthamoebic* keratitis. Antibody response was evaluated with IHA test on day 10 and 20 post inoculation. No antibody response was seen on day 10. However, slight antibody response was seen on day 20.
Leukocyte migration inhibition test (LMI) was performed to study the T-cell response in experimental *Acanthamoeba* keratitis in rabbits. The LMI was 40% on day 10 and 63% on day 20. The LMI response in the experimental animals did not show much variation among the Groups.

IHA antibody response were estimated in 200 human cases of which 20(10%) was from normal healthy controls. Among the others, 16 were *Acanthamoeba* keratitis cases. Out of these 16 cases, 14 were positive with high antibody titers.