# PATHOGENIC NAEGLERIA FROM THERMAL SPRINGS

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## Objectives

The long range goal of this research is to document the occurrence of pathogenic amoebae in thermal habitats that have been altered or disturbed by human activity. Immediate goals for this year included the comparison of filtration with centrifugation as a means of concentration of amoebae in water samples and testing the feasibility of using a commercially available enzyme detection system (API ZYM) for the identification of pathogenic Naegleria.

## Methods

Isolation of amoebae from filtered water samples has been successful but has the limitation of giving only an estimate of the number of organisms present. Centrifugation has the potential to better quantitate the population densities of amoebae. Forty-five milliliters of water sample were centrifuged at maximum speed on a table top clinical centrifuge for 10 minutes. The supernatant was withdrawn with a sterile pipet until approximately 1.0 mL remained. The concentrated sample was spread on the surface of non-nutrient agar plates along with Escherichia coli as a food source and incubated at 42 C.

When plaques appeared, a piece of agar with the amoebae was removed and transferred to a tissue culture flask containing 3.0 mL of sterile distilled water. These flasks were incubated at 37 C and observed at 2, 4 and 24 hours for the presence of flagellates. The flasks permitted observation of the morphology of the amoebae and the determination of the presence of flagellates without the need for preparing slides. It also serves well as a convenient transport container. Amoebae isolated at 42 C and forming flagellates at 37 C are considered to be Naegleria. They were then transferred to axenic growth medium.

The commercially available API ZYM system allows the assessment of the

activity of 19 hydrolytic enzymes. Following the methods of Kilvington and White (1985) and the manufacturer's instructions, axenically grown amoebae isolates were inoculated onto the API ZYM strips, incubated at 37 C and the results read after 4 hours.

The primary study site was the Huckleberry Hot Springs located just north of the Grand Teton National Park. The site consists of several springs that flow into Polecat Creek. Additional sites included commercially developed springs in the region.

## Results

Table 1 summarizes the amoebae isolations from June of 1987. Only a single Naegleria strain was isolated because of technical difficulties in adapting the centrifugation technique. However, it is significant to note that under the conditions of isolation used, a greater variety of other amoebae were isolated. Table 2 summarizes the July isolations after solving the technical problems. Naegleria represented nearly 37% of the total number of isolates. Preliminary identification of the Naegleria isolates by API ZYM is shown in Table 3.

## Conclusions

We have modified our methods for the isolation and identification of thermophilic amoebae. This method allows significant recovery of Naegleria. The API ZYM procedure has potential to decrease the time required for identification.

## Literature Cited

Kilvington, S. and D. G. White. 1985. Rapid identification of thermophilic Naegleria, including Naegleria fowleri using API ZYM system. J. Clin. Pathol. 38:1289-1292.

TABLE 1. Amoeba isolates (June, 1987)

| Site | Temp (C) | Total Plaques | Total Isolates | Identification |
|---|---|---|---|---|
| Huckleberry Hot Springs | | | | |
| Runoff Chanel | 42 | < 1 | <1 | |
| Artificial Pool | 42 | 16 | 16 | 16 Willaertia |
| Creek | 40 | < 1 | <1 | |
| Polecat Creek Springs | | | | |
| Campground Artificial Pool | 40 | 1 | 1 | NC* |
| Kelley Warm Springs | 29 | < 1 | <1 | |
| Commercial Springs #1 | | | | |
| Hotest Pool | 45 | 2 | 2 | 2 Acanthamoeba |
| Deep Well | 54 | < 1 | <1 | |
| Swimming Pool | 30 | < 1 | <1 | |
| Old Spring | 52 | < 1 | <1 | |
| Commercial Pool #2 | | | | |
| Swimming Pool | 39 | 1 | 1 | 1 Acanthamoeba |
| Spring | 39 | 27 | 15 | 15 Acanthamoeba |
| Commercial Pool #3 | | | | |
| Sediment Pool | 40 | < 1 | <1 | |
| Swimming Pool | 36 | 1 | 1 | 1 Hartmannella |
| Pool Effluent | 36 | 3 | 3 | 1 Naegleria<br>2 Hartmennella |
| Oldfaithful Springs | | | | |
| Pool #1 | 40 | 4 | 4 | NC |
| Pool #2 | 40 | 5 | 5 | NC |
| Boiling River | | | | |
| Upstream | 45 | 1 | 1 | 1 Acanthamoeba |
| Downstream | 34 | 2 | 2 | NC |
| Ranger Pool | 35 | 13 | 13 | 13 Vahlkampfia |
| Spirea Creek | | | | |
| Pool #1 | 43 | < 1 | <1 | |
| Pool #2 | 45 | < 1 | <1 | |

*Identification not completed

TABLE 2. Amoeba isolates (July, 1987)

| Site | Temp (C) | Total Plaques | Total Isolates | Naegleria Isolates |
|---|---|---|---|---|
| Huckleberry Hot Springs | | | | |
| Runoff Channel | 42 | 13 | 7 | < 1 |
| Artificial Pool | 41 | 21 | 16 | 1 |
| Creek | 40 | 7 | 7 | 2 |
| Commercial Springs #1 | | | | |
| Hotest Pool | 42 | 3 | 3 | < 1 |
| Swimming Pool Inlet | 32 | 15 | 15 | 11 |
| Old Springs | 40 | 21 | 20 | 16 |
| Commercial Pool #2 | | | | |
| Swimming Pool | 37 | < 1 | <1 | < 1 |
| Spring | 40 | 55 | 15 | 3 |
| Commercial Pool #3 | | | | |
| Sediment Pool | 40 | 3 | 3 | < 1 |
| Swimming Pool | 37 | 1 | 1 | < 1 |
| Pool Effluent | 37 | 5 | 5 | 1 |
| Ranger Pool | | | | |
| Pool | 33 | < 1 | <1 | < 1 |
| Stream | 45 | < 1 | <1 | < 1 |
| TOTAL | | 144 | 92 | 34 |

TABLE 3. *Naegleria* isolates completed and identified by API ZYM

| Site | Temp (C) | *N. lovaniensis* | *N. australiensis* |
|---|---|---|---|
| Huckleberry Hot Springs | | | |
| Artificial Pool | 41 | <1 | 1 |
| Commercial Springs #1 | | | |
| Swimming Pool Inlet | 32 | 4 | <1 |
| Old Spring | 40 | 3 | 7 |
| Commercial Pool #2 | | | |
| Spring | 40 | <1 | 2 |
| Commercial Pool #3 | | | |
| Pool Effluent | 37 | 1 | <1 |
| TOTAL | | 8 | 10 |