

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 improvement of isolation techniques and the field testing of a selective medium for the specific isolation of pathogenic Naegleria.

Methods

While centrifugation is better than filtration for the isolation of amoebae from water samples, it still has some limitations. Therefore, if samples contained soil or algae, they were mixed with a small amount of water and spotted on the surface of non-nutrient agar plates along with Escherichia coli as a food source and incubated at 42 C. Water samples were pipeted (10mL) onto the same type of plates and allowed to stand for one hour while the amoebae attached. The excess water was then carefully poured off before incubation.

When plaques appeared, they were transferred to a well of a tissue culture plate containing a suspension of E. coli in distilled water. These plates were incubated at 37 C and observed for growth and the presence of flagellates. The wells permitted observation of the morphology of the amoebae and the determination of the presence of flagellates without the need for preparing slides. Amoebae isolated at 42 C and forming flagellates at 37 C are considered to be Naegleria. They were then transferred to axenic growth medium.

Pentamidine isethionate has been shown to be selective against the growth of most amoebae while pathogenic Naegleria fowleri is resistant (Kilvington, 1988, personal communication). Pentamidine was incorporated in the non-nutrient agar as a selective agent.

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 the commercially developed springs in the region.

Results

The spotting of mud and algal samples and direct pipetting of water samples onto the surface of the plates were the best methods for isolation tested so far. They are simpler and more reproducible than filtration or centrifugation.

Pentamidine isethionate reduced significantly the numbers of interfering amoebae isolates. In one instance comparing treated and untreated samples, the drug reduced the number of isolates from 137 to 30. While laboratory strains of N. lovaniensis, a nonpathogenic, interfering species, are inhibited by the pentamidine, some field isolated strains are not.

The presence of N. australiensis was verified in several springs in the Grand Teton National Park area. This organism is pathogenic for mice but its potential for human infection has not been established. Pathogenic N. fowleri was not found in any of our samples.

Conclusions

We have modified our methods for the isolation and identification of thermophilic amoebae. These new methods allow significant recovery of Naegleria. Pentamidine isethionate is very useful in reducing the numbers of isolates that need to be screened for potential pathogens. The significance of the presence of N. australiensis needs to be established.