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# IN ARKANSAS RECREATIONAL WATERS

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# ABSTRACT

Selected recreational waters of Arkansas were sampled for pathogenic free-living limax amoebae. Water quality parameters were determined for correlation with amoebic population densities and species diversity. Cultural criteria and animal inoculation revealed no pathogenic strains. The feasibility of introduction and/or induction of pathogenic amoebic strains by environmental factors necessitates further ecological investigaitons.

## INTRODUCTION

Pathogenic strains of a free-living limax amoeba *Naegleria gruberi* cause primary amoebic meningoencephalitis (PAM), a fatal desease occurring in young adults or children after swimming in warm water with a high organic content. Fatalities are reported from several countries and from Georgia, Texas, Pennsylvania, Virginia, California, and Florida in the United States.

Drug therapy is ineffective in PAM; thus identification and closure of infective waters are presently the only preventive measures.

Symmers (1969) and Neva (1970) suggested PAM results from environmental pollution and emphasized the need for environmental studies. Duma et al. (1971) stated human meningoencephalitis resulting from environmental pollution may be a sizable problem, especially in the Southeastern United States. Griffin (1972) proposed thermal and coliform pollution promote growth of pathogenic *N. gruberi*. Therefore, a potentiality for the induction of pathogens exists through thermal or sewage effluents.

This study and others have attempted to isolate pathogenic *N. gruberi* and to correlate its presence and density with water quality (Nelson, 1972; Jamieson and Anderson, 1973).

## MATERIALS AND METHODS

Water samples were collected from selected Arkansas recreational waters during July and August of 1973 and 1974. A single sample from Dardanelle Reservoir was taken in November 1973. Water quality parameters were monitored by standard Hach field procedures. Coliform (excluding fecal coliform) were determined through Millepore membrane filtration.

Amoebae were sampled by Millipore membrane filtration methods. Five-μ filter membranes were washed repeatedly with 5 ml sterile distilled water prior to plating one- and three-drop samples on buffered sucrose tryptose agar with Pseudomonas aeruginosa (Chang, 1971). Plates were incubated at 35C for enumeration of total amoebic densities, or 41C for enumeration of pathogens. After 24-48 hr the 35C plates were incubated at room temperature. Amoebic plaques were counted at 3, 10, and 16 days. Organisms were identified by cultural and morphological criteria (Page, 1967; Chang, 1971, 1972, 1974). Selected plaques of amoebae were cultured on BST agar slants at 35C before intranasal inoculation of white mice to determine pathogenicity.

Isolation, inoculation, and identification phases of the study were conducted in facilities provided be the Division of Laboratory Animal Medicine, School of Medicine, University of Arkansas Medical Center, Little Rock, Arkansas.

## RESULTS

Water quality parameters, amoebic population densities, and species composition for each collection site are presented in Table I.

The average number of amoebae for all sites was 457/liter. The average species composition for the sites was: Naegleria gruberi 56.4%, Acanthamoeba rhysodes 35.0%, Hartmannella sp. 4.5%, and Schizopyrenus russelli 4.1%.

The highest amoebic density (699/l) was at Goshen Bridge and the lowest density (233/l) was at Horsehead Lake, excluding the seasonally induced low density at Dardanelle Reservoir. N. gruberi was the predominant species at all sites except Goshen Bridge. A. rhysodes was relatively abundant at all sites and was predominant at Goshen Bridge. S. russelli and Hartmannella sp. were found infrequently (Table I).

Water quality parameters were found to be at acceptable levels for primary contact recreational water. The water at Goshen Bridge showed greater pollution which may account for the high average total amoebic level (Table I).

No pathogenic amoebae were found based on cultural or morphological criteria.

Amoebic plaques failed to appear on the 41C plates used for selective growth of pathogens through temperature tolerance. Amoebic plaques also failed to appear on the 35C plates after incubation of 2 wk. Growth under these conditions would have indicated the presence of pathogenic free-living strains of Naegleria. The intranasal inoculation of white mice with selected strains of amoebae identified as nonpathogenic by cultural characteristics failed to demonstrate pathogenicity. No inoculated mice died and all animals appeared healthy during a 3 wk postinfection period.

## DISCUSSION

The number, species composition, and dominance of amoebae in the samples approximate other reported levels (Chang, 1971, 1972). Chang (1971) found Acanthamoeba better adapted to adverse conditions than Naegleria, thus the prominence of A. rhysodes at Goshen Bridge. N. gruberi was predominant at the other sites.

Little additional correlation of amoebic densities and composition is apparent, other than increasing popultion density with increasing water temperature. Water qualtiy probably influences the dynamics of amoebae synergistically.

Other investigators have attempted to isolate pathogenic free-living amoebae from nondisease-connected sources. Nelson (1972) isolated pathogenic Naegleria from a small, nonrecreational pond. The pathogenic strain was one of 226 cultivated strains.

Jamieson and Anderson (1973) cultured 130 strains from 400 sources and identified two pathogenic strains. Chang (1972) reported amoebic popultion levels from 15 sources. Although numerous strains with high densities were found, all amoebae were nonpathogenic.

These reports indicate pathogenic amoebae exhibit a low population density and are a small fraction of amobic isolates from nondisease areas.

The relationship of environmental pollution and pathogenic amoebae is obscure. Griffin (1972) showed pathogenic Naegleria and Acanthamoeba grew above 37C. Chang (1972) demonstrated nonpathogenic Naegleria grew in a simulated natural aquatic environment at 25C while pathogenic strains decreased rapidly. Chang did not dismiss the possibility of the extended survival of pathogenic Naegleria in a natural habitat under certain conditions.

Water quality in the present study did not favor the survival and/or growth of pathogenic amoebae, hence the apparent absence of pathogenic strains.

A human carrier state may contribute to the occurrence of PAM (Skocil et al., 1971; Chang, 1972, 1974). A carrier state is of epidemiological significance by the introduction of pathogens in uninfected waters, or disease induction in a carrier by certain water quality parameters.

Although no pathogenic amoebae were isolated during the present study, the potential of PAM cannot be discounted. The introduction of pathogenic amoebae in uninfected waters by human carriers or other unknown hosts coupled with a favorable environment, such as thermal effluents from thermonuclear reactors, is a situation for further investigation to answer the question posed by Neva (1970), "Is this another example of a new disease pattern that man creates by fouling his environment?"

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TABLE I. Water quality parameters and amoebae levels of Arkansas recreational waters.

| Site | Date | Temp °F | DO mg/l | CO$_2$ mg/l | pH | NO$_2$ mg/l | NO$_3$ mg/l | O-PO$_4$ mg/l | M-PO4 mg/l | Hardness gr/gal | Coliform #/100 ml | Avg. *N.g.* /l (%) | Avg. *A.r.* /l (%) | Avg. *H.sp.* /l (%) | Avg. *S.r.* /l (%) | Avg. Total Amoebae/l |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L. Weddington | 7-73 | 88.6<br>(88-89) | 8.3<br>(8-9) | 25<br>(10-40) | 8.6<br>(8.5-8.75) | 0 | 0 | .2<br>(0-.5) |  | 4.3<br>(4-5) | 1<br>(<1-1) | 377(69) | 178(31) |  |  | 555 |
| BEAVER RES. |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Lost Bridge | 7/8-73 | 88.5<br>(88-89) | 7.5<br>(7-8) | 52.5<br>(50-55) | 8.75 | 0 | 0 | 0 | 0 | 3 | 1<br>(<1-1) | 300(65) | 148(31) | 19(4) |  | 467 |
| Rocky Branch | 7/8-73 | 88<br>(87-89) | 7.5<br>(7-8) | 25.0<br>(20-30) | 8.75 | 0 | 0 | .25<br>(.3-.2) | .25<br>(.3-.2) | 3 | <1 | 208(53) | 169(42) | 22(5) |  | 399 |
| Prairie Creek | 7/8-73 | 86.8<br>(82-90) | 8.0<br>(7-9) | 25.0<br>(10-45) | 8.70<br>(8.5-8.75) | 0 | 0 | .06<br>(0-.3) | .1<br>(0-.3) | 3.2<br>(3-4) | 1<br>(<1-1) | 373(58) | 213(31) | 60(9) | 13(2) | 659 |
| Horseshoe Bend | 7/8-73 | 86.8<br>(84-89) | 8.2<br>(7-9) | 25.0<br>(10-45) | 8.75 | 0 | 0 | .2<br>(0-.5) | .2<br>(0-.5) | 3 | <1<br>(<1-1) | 360(52) | 226(33) | 46(7) | 57(8) | 689 |
| Monte Ne | 7/8-73 | 87.5<br>(87-88) | 8.5<br>(8-9) | 42.5<br>(40-45) | 8.5 | 0 | 0 | 0 | 0 | 3 | 1<br>(<1-1) | 533(81) | 116(18) | 11(1) |  | 660 |
| Hickory Creek | 7/8-73 | 84.8<br>(82-86) | 8.2<br>(7-9) | 25.0<br>(10-35) | 8.3<br>(7.8-8.75) | 0 | 0 | .2<br>(0-.5) | .12<br>(0-.5) | 3.4<br>(3-5) | <1<br>(<1-1) | 401(59) | 199(29) | 33(5) | 56(7) | 689 |
| Goshen Bridge | 7-73 | 82.6<br>(79-86) | 9.3<br>(4-15) | 71.6<br>(30-125) | 7.2<br>(7-7.5) | .39<br>(.2-.8) | .51<br>(.2-.8) | 1.0<br>(.3-1.5) | .8<br>(0-1.2) | 3.7<br>(3-4) | 2<br>(1-3) | 300(42) | 311(45) | 72(11) | 16(2) | 699 |
| Dardanelle Res. | 11-73 | 63 | 8 | 65 | 8.5 | 0 | .5 | 0 | 0 | 5 | <1 | 10(27) | 26(73) |  |  | 36 |
| Dardanelle Res. | 7-74 | 88 | 7 | 35 | 8.5 | 0 | 0 | 0 | 0 | 3 | 2 | 233(61) | 125(33) | 25(6) |  | 383 |
| Shores L. | 7-74 | 89 | 8 | 20 | 8.7 | .1 | .3 | .2 | .6 | 5 | 1 | 300(75) | 100(25) |  |  | 400 |
| Horsehead L. | 7-74 | 90 | 8 | 30 | 8.5 | .1 | .2 | .1 | .2 | 4 | 2 | 100(42) | 100(42) | 33(16) |  | 233 |
| Atkins L. | 7-74 | 89 | 8 | 20 | 8.5 | .3 | .2 | .4 | .3 | 4 | 1 | 283(60) | 100(21) | 84(19) |  | 467 |
| L. Conway | 7-74 | 89 | 8 | 45 | 8.5 | .3 | .5 | .7 | .9 | 4 | 3 | 293(61) | 117(24) | 17(6) | 47(9) | 474 |
| Big Maumelle L. | 7-74 | 88 | 9 | 25 | 8.7 | 0 | 0 | 0 | 0 | 3 | <1 | 118(33) | 233(66) |  | 33(7) | 616 |
| Harris Brake | 7-74 | 87 | 8 | 15 | 8.7 | .3 | .5 | .3 | .3 | 4 | 1 | 350((56) | 233(37) |  | 33(7) | 616 |
| L. Winona | 7-74 | 88 | 9 | 10 | 8.7 | 0 | 0 | 0 | 0 | 3 | 2 | 118(50) | 118(50) |  |  | 236 |
| Nimrod L. | 7-74 | 89 | 8 | 25 | 8.7 | 0 | 0 | 0 | 0 | 5 | <1 | 250(65) | 100(26) |  | 33(9) | 383 |
| Bl. Mountain L. | 7-74 | 90 | 8 | 30 | 8.7 | 0 | 0 | 0 | 0 | 3 | <1 | 188(61) | 117(39) |  |  | 305 |
| L. Wilhelmina | 7-74 | 89 | 7 | 35 | 8.5 | .7 | 1.0 | .3 | .2 | 4 | 3 | 300(50) | 200(33) |  | 100(17) | 600 |
| DeQueen L. | 7-74 | 88 | 8 | 10 | 8.5 | 0 | 0 | 0 | 0 | 2 | 4 | 284(54) | 150(29) |  | 82(17) | 516 |
| Gillham L. | 7-74 | 87 | 8 | 20 | 8.5 | 0 | 0 | 0 | 0 | 5 | 3 | 318(72) | 117(28) |  |  | 435 |
| Dierks L. | 7-74 | 88 | 8 | 45 | 8.5 | 0 | 0 | 0 | 0 | 4 | <1 | 233(50) | 200(42) | 33(8) |  | 466 |
| Shady L. | 7-74 | 87 | 8 | 45 | 8.7 | .1 | .3 | .05 | .1 | 4 | 4 | 250(46) | 250(46) |  | 33(8) | 533 |
| L. Greeson | 7-74 | 89 | 7 | 15 | 8.5 | 0 | 0 | 0 | 0 | 2 | 2 | 300(78) | 83(22) |  |  | 383 |
| Millwood L. | 7-74 | 88 | 9 | 40 | 8.5 | .2 | .3 | .2 | .2 | 5 | 1 | 233(63) | 100(27) | 33(10) |  | 366 |
| DeGray L. | 7-74 | 86 | 8 | 40 | 8.7 | 0 | 0 | 0 | 0 | 4 | 2 | 200(46) | 200(46) | 17(4) | 16(4) | 433 |
| L. Hamilton | 7-74 | 86 | 8 | 10 | 8.5 | .4 | .3 | .2 | .6 | 5 | <1 | 200(48) | 106(25) | 27(6) | 83(21) | 416 |
| L. Quachita | 7-74 | 87 | 9 | 15 | 8.5 | 0 | 0 | 0 | 0 | 3 | 2 | 283(85) | 33(10) |  | 17(5) | 333 |
| L. Catherine | 7-74 | 86 | 8 | 30 | 8.7 | .1 | .2 | .05 | .1 | 4 | 2 | 200(46) | 200(46) | 33(8) |  | 433 |
| Greers Ferry | 7-74 | 88 | 8 | 25 | 8.5 | 0 | 0 | 0 | 0 | 3 | 1 | 166(46) | 133(36) | 33(9) | 33(9) | 365 |
| Buffalo R. | 7-74 | 86 | 7 | 35 | 8.5 | .1 | .1 | .05 | .05 | 5 | <1 | 350(68) | 250(42) |  |  | 600 |
| Norfork L. | 7-74 | 89 | 8 | 20 | 8.7 | .2 | .3 | .1 | .2 | 2 | 2 | 233(58) | 117(29) | 33(8) | 18(5) | 401 |
| Bull Shoals | 7-74 | 87 | 9 | 40 | 8.7 | .1 | .2 | .1 | .3 | 3 | 3 | 266(47) | 200(35) | 66(11) | 33(7) | 565 |