Journal of the Arkansas Academy of Science

Volume 29 Article 7

1975

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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 possibility of introduction and/or induction of pathogenic amoebic strains by environmental factors requires further ecological investigations.

INTRODUCTION

Pathogenic strains of a free-living limax amoeba, *Naegleria gruberi*, cause primary amoebic meningoencephalitis (PAM), a fatal disease 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 is now the only preventive measure.

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 sizeable problem, especially in the Southeastern United States. Griffin (1972) proposed thermal and coliform pollution promoted growth of pathogenic *N. gruberi*. Therefore, there is a potentiality for the induction of pathogens through thermal or sewage effluents.

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

MATERIALS AND METHODS

Water samples were collected from selected recreational waters during July and August 1973-74. A single sample from Dardanelle Reservoir was taken in November 1973. Water quality parameters were monitored by standard Hach field procedures.

Subsurface water samples for physicochemical and organismic analyses were taken within 1 m of the shore. Designated swimming areas were selected as collecting sites. Recreational waters without swimming areas per se were sampled at readily accessible locations, such as boat launching sites.

Amoebae were sampled by Millipore membrane filtration methods. Filter membranes of 5μ porosity were washed repeatedly with 5 ml sterile distilled water before plating of one- and three-drop samples on buffered sucrose tryptose agar (BST) 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 (Chang, 1971, 1972, 1974; Page, 1967). Selected plaques of amoebae were cultured on BST agar slants at 35C before intranasal inoculation in white mice for determination of pathogenicity.

Isolation, inoculation and identification phases of this study were conducted in facilities provided by 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 shown 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%, *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 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 waters. The water at Goshen Bridge showed higher CO_2, nitrite and nitrate levels and a lower pH which may account for the high average total amoebae population level (Table I).

No pathogenic amoebae were found on the basis of 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 indicate 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 any pathogenicity. No deaths occurred in inoculated mice 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 population density with increasing water temperature. Water quality parameters probably act synergistically on the population dynamics of amoebae.

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 population levels from 15 sources. Although numerous strains with high densities were found, all amoebae were nonpathogenic.

These reports indicate pathogenic amoebae are low in population density and constitute a small fraction of amoebic isolates from nondisease areas.

The relationship of environmental pollution and pathogenic amoebae is obscure. Griffin (1972) showed pathogenic *Naegleria* and *Acanthamoeba* grew at temperatures above 37C. Chang (1972) demonstrated nonpathogenic *Naegleria* grew in a simulated natural aquatic environment at 25C whereas 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 (Chang, 1972, 1974; Skocil et al., 1971). A carrier state offers epidemiological significance through 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?"

ACKNOWLEDGEMENTS

This project was supported with funds provided by the Office of Water Research and Technology, U.S. Department of the Interior, under Grant Number A-028-ARK as authorized under the Water Resources Research Act of 1964, P.L. 88-379 as amended by P.L. 89-404 and P.L. 92-175.

Special appreciation is extended to Dr. Harold E. Farris, Director, Division of Laboratory Animal Medicine, School of Medicine, University of Arkansas Medical Center, Little Rock, Arkansas, for his splended cooperation in the use of laboratory facilities.

LITERATURE CITED

CHANG, S. 1971. Small, free-living amoebas: cultivation, quantitation, identification, classification, pathogenesis, and resistance. Current Topics in Comp. Pathobiol. 1: 201-254.

CHANG, S. 1972. Pathogenic free-living amoebae and recreational waters. Presented at 6th Conf. Int. Water Pollution Research Assoc., June 18-23, 1972, Jerusalem, Israel.

CHANG, S. 1974. Etiological, pathological, epidemiological, and diagnostical considerations of primary amoebic meningoencephalitis. CRC Crit. Rev. Microbiol. 3: 135-159.

DUMA, R., W. ROSENBLUM, R. McGEHEE, M. JONES and E. NELSON. 1971. Primary amoebic meningoencephalitis caused by *Naegleria*. Ann. Int. Med. 74: 861-869.

GRIFFIN, J. 1972. Temperature tolerance of pathogenic and nonpathogenic free-living amoebas. Science 178: 869-870.

JAMIESON, A., and K. ANDERSON. 1973. A method for isolation of *Naegleria* species from water samples. Pathology 5: 55-58.

NELSON, E. 1972. Procedures for the isolation of pathogenic *Naegleria*. Va. J. Sci. 23: 145 (Abstr.)

NEVA, F. 1970. Amebic meningoencephalitis - a new disease? New England J. Med. 282: 450-452.

PAGE, F. 1967. Taxonomic criteria for limax amoebae, with descriptions of 3 new species of *Hartmannella* and 3 of *Vahlkampfia*. J. Protozool. 14: 499-521.

SKOCIL, V., L. CERVA and C. SERBUS. 1971. Epidemiological study of amoeba of the limax group in military communities: relations between the findings of amoeba in the external environment and their incidence in soldiers. J. Hyg. Epidemol. Microbiol. Immunol. (Prague) 15: 445-449.

SYMMERS, W. 1969. Primary amoebic meningoencephalitis in Britain. Brit. Med. J. 4: 449-454.

Table I. Water Quality Parameters and Amoebae Population Levels* (Sites Lost Bridge through Goshen Bridge are on Beaver Reservoir)

Site	Date	Temp °F	DO mg/l	CO_2 mg/l	pH	NO_3 mg/l	NO_2 mg/l	O-PO_4 mg/l	T-PO_4 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 (88-89)	8.3 (8-9)	25 (10-40)	8.6 (8.5-8.75)	0	0	.2 (0-.5)		4.3 (4-5)	1 (<1-1)	377(69)	178(31)			555
Lost Bridge	7/8-73	88.5 (88-89)	7.5 (7-8)	52.5 (50-55)	8.75	0	0	0	0	3	1 (<1-1)	300(65)	148(31)	19(4)		467
Rocky Branch	7/8-73	88 (87-89)	7.5 (7-8)	25.0 (20-30)	8.75	0	0	.25 (.3-.2)	.25 (.3-.2)	3	<1	208(53)	169(42)	22(5)		399
Prairie Creek	7/8-73	86.8 (82-90)	8.0 (7-9)	25.0 (10-45)	8.70 (8.5-8.75)	0	0	.06 (0-.3)	.1 (0-.3)	3.2 (3-4)	1 (<1-1)	373(58)	213(31)	60(9)	13(2)	659
Horseshoe Bend	7/8-73	86.8 (84-89)	8.2 (7-9)	25.0 (10-45)	8.75	0	0	.2 (0-.5)	.2 (0-.5)	3	<1 (<1-1)	360(52)	226(33)	46(7)	57(8)	689
Monte Ne	7/8-73	87.5 (87-88)	8.5 (8-9)	42.5 (40-45)	8.5	0	0	0	0	3	1 (<1-1)	533(81)	116(18)	11(1)		660
Hickory Creek	7/8-73	84.8 (82-86)	8.2 (7-9)	25.0 (10-35)	8.3 (7.8-8.75)	0	0	.2 (0-.5)	.12 (0-.5)	3.4 (3-5)	<1 (<1-1)	401(59)	199(29)	33(5)	56(7)	689
Goshen Bridge	7-73	82.6 (79-86)	9.3 (4-15)	71.6 (30-125)	7.2 (7-7.5)	.39 (.2-.8)	.51 (.2-.8)	1.0 (.3-1.5)	.8 (0-1.2)	3.7 (3-4)	2 (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. Ouachita	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

*Coliform numbers exclude fecal coliform.

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