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Agglutination of *Naegleria fowleri* by Human Serum[1] (41420)

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Abstract. The capability of 154 serum samples from pediatric outpatients and 101 samples from adults to agglutinate amoebae of *Naegleria fowleri* nN68 was assessed. Sera from all 19 infants tested had an agglutination titer of 1:4 or less; sera of toddlers had a median agglutination titer of 1:8 and those of adults, 1:16. Only 13 of 154 serum samples from children had titers of 1:32 or 1:64; 7 of these high titered sera were from the 38 asthmatic children. Selected sera were found to give comparable titers when assayed against eight other strains of *N. fowleri*. Agglutination activity was removed by absorption with *N. fowleri* but not by absorption with *N. gruberi*. The agglutinating activity was specific for the human pathogenic *N. fowleri*. The ubiquitous free-living *N. gruberi* was not the immunogen responsible for the agglutinating activity in human serum.

Primary amoebic meningoencephalitis is a rare, acute, usually fatal disease of active juveniles (1). The disease has been encountered throughout the world, and the etiologic agent *Naegleria fowleri* has been isolated from a variety of aquatic environments in both hemispheres (2, 3). Antibodies reacting with *N. fowleri* have been detected in pooled sera from young adult women in the United States using a sensitive radioimmune assay (4) and in serum samples from adults and infants in New Zealand (5). The antibody titers in the latter survey, obtained by a sensitive indirect fluorescent antibody assay, were 1:10 for all adult sera and 1:5 for all cord blood samples. The antibody titers reported by Cursons *et al.* (5) for the New Zealand serum samples were lower than anticipated from the radioimmune assay of Tew *et al.* (4). Moreover, the antibody titers reported for the New Zealand serum samples were more uniform than expected for unwitting exposure to amoebae.

We have undertaken a survey of the antibody levels against *N. fowleri* of individual sera from a variety of healthy adults and pediatric outpatients. The agglutination test, introduced by Anderson and Jamieson (6) was used to assay antibody activity. The objectives of this study have been to determine whether agglutination titers against *N. fowleri* are low and uniform or quite variable among different individuals, and whether antibody activity is strain or species specific. Agglutination titers have also been examined for possible correlations with sex, age, or medical history of the subjects.

The agglutination of 255 human serum samples ranged from less than 1:2 to 1:64. Sera from infants less than 1 year old had agglutination titers less than 1:4 whereas sera from young adults had a median titer of 1:16.

Materials and Methods. *N. fowleri* strains, obtained from various sources (7), were grown in Nelson medium composed of 1 g liver digest (Panmede, Harrison and Crosfield, Bronxville, N.Y.), 1 g glucose, 0.12 g NaCl, 0.142 g Na_2HPO_4, 0.136 g KH_2PO_4, 0.004 g $MgSO_4 \cdot 7H_2O$, and 0.004 g $CaCl_2 \cdot 2H_2O$ in one liter of deionized water, and supplemented with 2% (v/v) calf serum (Gibco, Grand Island, N.Y.). *N. gruberi* EGB was grown in Balamuth medium composed of 5 g glucose, 5 g yeast extract, 10 g liver digest, 10 g proteose peptone, 0.12 g NaCl, 0.142 g Na_2HPO_4, 0.136 g KH_2PO_4, 0.004 g $MgSO_4 \cdot 7H_2O$, and 0.004 g $CaCl_2 \cdot 2H_2O$ in one liter of deionized water (7). Filter sterilized hemin was added to a final concentration of 2 μg/ml (8).

Samples of fresh human serum were ob-

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tained from the Pediatric Outpatient Clinic, Medical College of Virginia Hospitals, Richmond, Virginia, and from medical students at Virginia Commonwealth University, Richmond, Virginia. The sera were filtered through membrane filters (0.45-μm pore size) and stored at −20° until used. Complement was inactivated by heating at 56° for 30 min.

Amoebae for agglutination assays were harvested from axenic cultures and suspended in Eagle's minimum essential medium (9). The suspension was chilled to 5° and diluted to give 1×10^6 amoebae/ml. The amoebae were mixed with serum dilutions in microtiter plates (B-D Immunodiagnostics, Oxnard, Calif.) and then incubated at 37° for 30 min. Agglutination was scored by examining the amoeba suspensions with an inverted compound light microscope. Agglutination titers were expressed as the greatest serum dilution capable of agglutinating the amoebae.

Results. The agglutinating capability of 154 human serum samples from infants, children, and juveniles, and from 101 adults was assessed. The sera of all 19 subjects less than 1 year old had agglutination titers against *N. fowleri* nN68 of 1:4 or less. The sera of 17 subjects 1 to 2 years old had a median agglutination titer of 1:8; the serum from two toddlers agglutinated amoebae of *N. fowleri* nN68 at a dilution of 1:16. Sera of the 101 adults had a median agglutination titer of 1:16; the serum from 20 of the adults had agglutination titers of 1:64. One adult (56 years old) had an agglutination titer of 1:2 or less. There was no marked correlation between sex and agglutination titer for any age group (Table I).

The agglutinating titers of the 154 serum samples from the pediatric outpatients were also arranged according to symptoms or diagnosis noted upon examination in the clinic. Only 13 of 154 serum samples from pediatric outpatients possessed capability to agglutinate *N. fowleri* nN68 at a dilution of 1:32 or 1:64. Seven of the samples with high agglutination titers were from the 38 asthmatic children. Sera from pediatric outpatients with asthma had agglutination titers slightly, but consistently, higher than those of sera from other subjects of like age. Two of the sera with agglutination titers of 1:32 or 1:64 were from patients with brain involvements (a 10 year old with persistent headaches and a 17 year old with seizures). The remaining four sera with titers of 1:32 or 1:64 were from patients with miscellaneous symptoms (one bee sting, one hyperventilation, one gonorrhea, one abdominal pain). Sera from the two mentally retarded individuals and two leukemia patients had negligible agglutinating capability. Children suffering from fever, sepsis, or respiratory distress were younger than those presenting other symptoms (Table II), but their agglutination titers were the

TABLE I. CAPABILITY OF HUMAN SERUM SAMPLES TO AGGLUTINATE AMEBAE OF *Naegleria fowleri* nN68

Age group	No. subjects		No. with agglutinating titer of													
			<1:2		1:2		1:4		1:8		1:16		1:32		1:64	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0–4 months	2	4	2	4												
4–12 months	6	7	3	3	2	3	1	1								
1–2 years	9	8	1	1	2	2	1	1	3	4	2	0				
2–4 years	6	8			1	1	1	3	3	3	1	1				
4–6 years	10	9					0	3	6	4	2	3	2	0		
6–8 years	7	4			1	1	1	0	1	2	2	1	1	0	1	0
8–10 years	6	3					1	0	0	1	4	1	1	1		
10–15 years	30	14			0	1	2	1	16	3	10	6	0	0	2	2
15–20 years	10	11			1	0	0	1	2	5	6	3	1	1	0	1
20 years and older	8	12					1	2	0	1	5	6	2	2		
Medical students	(81)						(4)		(9)		(31)		(23)		(14)	

TABLE II. SYMPTOMS OF PEDIATRIC OUTPATIENTS AND THE AGGLUTINATING CAPABILITY OF THEIR SERA

Symptom	Subjects		No. with agglutinating titers of						
	No.	Median age	<1:2	1:2	1:4	1:8	1:16	1:32	1:64
Asthma	38	11 years			4	11	16	4	3
Brain involvement	15	4 years	2	1	2	7	1	1	1
Fever and sepsis	18	10 months	9	2	2	3	2		
Hematologic disorder	9	10 years		2	1	2	4		
Mental retardation	2	7, 20 years		2					
Respiratory distress	5	15 months		2	2	1			
Miscellaneous	38	9 years	2	5	3	15	9	2	2
Unknown	29	13 years	1	1	3	14	10		
Total population	154	7 years	14	15	17	53	42	7	6

same as those for all subjects of like age (Table I).

The specificity of the agglutinating capability of the human serum samples was assessed. Sera with agglutinating titers of 1:16 or 1:32 with respect to *N. fowleri* nN68 had comparable titers when assayed against *N. fowleri* strains HB-4, 6088, NF66, Lovell, 0359, GJ, TY, and WM. Similarly, sera with agglutinating titers of 1:8 had comparable titers when assayed against these eight strains. Sera with low agglutinating capability against strain nN68 were also unable to agglutinate other strains of *Naegleria*. Agglutination titers were markedly less when strain KUL or CJ was used in the assay. *N. gruberi* EGB was not agglutinated at dilutions greater than 1:4 by any of the tested human sera. Agglutinating activity for nN68 was removed by absorption with nN68, KUL, and CJ but not by absorption with *N. gruberi* EGB.

Discussion. Sera from human infants have negligible capability to agglutinate *N. fowleri*. Infants develop agglutinating activity beginning with the fourth month. Agglutinating activity rises progressively during the next several years. By age 4 years, essentially all of the children have measurable agglutinating activity. The agglutinating activity is specific for *N. fowleri*, therefore the ubiquitous free-living *N. gruberi* is not the immunogen responsible for agglutinating activity. Other workers have failed to detect cross-reacting antigens between *N. fowleri* and *Acanthamoeba, Entamoeba,* or *Hartmannella* (10, 11); therefore these amoebae are probably not the immunogen eliciting *N. fowleri* agglutinating activity. It is possible that *N. fowleri* itself is the immunogen responsible for this immune response. If so, essentially all children are exposed to sufficient *N. fowleri* to elicit an immune response. Exposure to *N. fowleri* would appear to lead to progressive primary amoebic meningoencephalitis only rarely. It is conceivable that infection with *N. fowleri* is the cause of subclinical or mild diseases whose etiology has not been established. It is possible that juveniles who have not been previously exposed to *N. fowleri* antigens and/or who have not developed antibodies against *N. fowleri* are vulnerable to primary amoebic meningoencephalitis. The epidemiology of primary amoebic, meningoencephalitis has some parallels with that of paralytic poliomyelitis prior to the development of effective immunization regimens. Paralytic poliomyelitis occurred most often in the theretofore healthy juvenile of economically middle-class parents (12).

The higher titers against *N. fowleri* observed in asthmatic children may reflect enhanced exposure to airborne antigen because of the airway obstruction characteristic of this disease or some elusive hyperreactivity to airborne antigen (13). For example, free-living amoebae have been implicated in humidifer disease, an allergic response to vapor-borne allergens (14). Other observations that merit further attention are the low agglutinating activity of sera from mentally retarded patients and elevated agglutination titers of the two individuals with brain involvement. The former

may reflect restricted exposure to environmental immunogen.

There have been only two prior reports on antibody in human serum directed against *N. fowleri*. Tew *et al.* (4) demonstrated that a single pooled serum sample from nursing students possessed antibodies directed to both surface antigens and internal constituents. Cursons *et al.* (5) conducted a survey in New Zealand for antibodies against *Naegleria* and other amoebae in adult and infant (cord blood) sera. The activities measured were low, and showed very little variation. In contrast, we have observed that agglutination titers for adult sera range from 1:2 to 1:64. It seems unlikely that these differences reflect geographic differences, rather differences in the immunologic assays.

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