Background Prevalence of Microagglutination Antibodies to *Legionella pneumophila* Serogroups 1, 2, 3, and 4

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The background prevalence of microagglutination antibodies to *Legionella pneumophila* was determined by testing the sera of 517 individuals who lived or worked in a small Iowa town. In this population, the upper limit of normal microagglutination titer for serogroups 1, 3, and 4 was 1:16, and that for serogroup 2 was 1:8. The prevalence of microagglutination titers of $\geq 1:32$ against any serogroup of *L. pneumophila* was only 7.4% and did not vary significantly with age or sex.

The microagglutination (MA) antibody assay has proved to be a valuable tool in diagnosing legionellosis (1, 5). Knowledge of the background prevalence of MA antibodies to *Legionella pneumophila* is crucial to the correct interpretation of serological results. Thus far, the prevalence of MA antibodies has been determined for serogroup 1 organisms only (5). We report here the prevalence of MA titers against *L. pneumophila* serogroups 1, 2, 3, and 4 in sera from 517 volunteers from a small rural town.

Sera. Sera were donated in September 1978 by 517 volunteers from a town of 800 people in an agricultural area of northeast Iowa. Volunteers ranged in age from 2 to 90 years; 249 were male and 268 were female. Of these, 89% were residents, and 78% worked in the community. None of the volunteers were acutely ill. Conversations with two local physicians and an analysis of questionnaires disclosed no unusual prevalence of respiratory illness in the community, although 6 weeks before samples were obtained, a local resident with *L. pneumophila* serogroup 1 pneumonia had been hospitalized elsewhere.

Blood was collected aseptically in sterile Vacutainer tubes (Becton, Dickinson & Co., Rutherford, N.J.) and allowed to clot overnight at 5°C. Tubes were centrifuged, and the sera were collected and stored in 1-ml samples in screw-cap glass vials at -60°C. Sera were assayed within 4 months of storage.

MA test. The MA test was performed by the procedure of Farshy et al. (1). *L. pneumophila* serogroups 1 (Philadelphia 1), 2 (Togus), 3 (Bloomington), and 4 (Los Angeles) were grown

on enriched Mueller-Hinton agar (3), harvested with sterile phosphate-buffered saline (pH 7.2), and washed twice. Heat-killed bacteria were suspended to an optical density of 0.65 at 420 nm.

Serial twofold dilutions of serum in phosphate-buffered saline (pH 7.2) were prepared with 0.05-ml microtiter diluters (Cooke Engineering Co., Alexandria, Va.) in rigid polystyrene plates (Linbro Scientific Co. Dublin, Va.). A 0.05-ml volume of antigen suspension was added to each well. The plates were then incubated overnight at room temperature and refrigerated for 2 h at 4°C. The MA titer was the highest dilution of serum resulting in a mat of bacteria without button formation.

The distribution of serum MA antibody titers against *L. pneumophila* serogroups 1, 2, 3, and 4 in the sera is shown in Table 1. The upper limit of the normal MA titer for *L. pneumophila* serogroup 1, serogroup 3, and serogroup 4 was 1:16 since that titer was exceeded by fewer than 15% of test sera (4). The upper limit of normal titer for serogroup 2 was 1:8, only a twofold dilution less.

The number of sera containing MA antibodies against more than one serogroup of *L. pneumophila* at titers of equal to or greater than the upper limit of normal was significant. About one-quarter (23%) had MA titers of $\geq 1:16$ against more than one serogroup. Only two sera, however, had titers of $\geq 1:32$ against two or more serogroups.

High MA titers ($\geq 1:32$) against the four serogroups of *L. pneumophila* were uncommon. Only 1.6, 0.8, 3.5, and 2.1% of the sera contained titers of $\geq 1:32$ against serogroups 1, 2, 3, and 4, respectively.

A total of 38 sera contained high MA titers against one or more of the antigens tested, for a

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frequency of 7.4%. In Table 2, the age and sex frequencies of these high-titered sera are shown. There were no significant differences in prevalence with age stratification, although the 3% frequency in individuals 60 years of age and older appeared to be two to three times less than that in other age groups. A 2-year-old boy and 87-year-old woman were the youngest and oldest individuals with MA antibody titers of $\geq 1:32$. The prevalence of high titers in males (6.0%) and in females (8.6%) was not significantly different.

The upper limit of normal MA titer for *L. pneumophila* serogroup 1 (Knoxville) has been estimated previously at 1:8 in tests on 830 blood donor, streptococcal, and febrile sera (5). The upper limit of normal value of 1:16 found for serogroup 1 (Philadelphia 1) in the present study of 517 sera from a rural population is similar to the previously reported value, differing by only one twofold dilution. This minor difference may be real, but it may also be accounted for by our use of a different strain of serogroup 1 organisms in the MA assay. The upper limit of normal titers for the three remaining serogroups of *L. pneumophila* was similar to that for serogroup 1.

Serum MA antibodies to each of the four serogroups of *L. pneumophila* were found in the population. Moreover, 23% of sera examined contained antibodies at titers of $\geq 1:16$ against two or more of the serogroups. At face value, such data suggest that the community and in-

dividuals were exposed to multiple serogroups of *L. pneumophila*. However, since *L. pneumophila*, *Legionella*-like organisms, and other gram-negative bacteria may share antigens (2, 7, 8), the possibility that cross-reacting antibodies might account for these findings cannot be excluded.

High titers of MA antibodies ($\geq 1:32$) occurred infrequently in the population. The prevalence of such high titers did not vary when samples were stratified by age or sex, which suggests that the rate of *L. pneumophila* infection, and possibly exposure, is similar for both sexes and for all age groups. It is known that Legionnaires disease pneumonia occurs more frequently in males and in individuals over 40 years of age (6). Perhaps secondary factors related to age and sex account for the increased prevalence of disease in males and in individuals over 40 years of age.

The present study confirms the rationale of the previous suggestion (5) that a titer of 1:32 be the lowest suggestive of infection, not necessarily of disease. In this rural setting, in a case of suspect illness, the specificity of a single convalescent MA titer of $\geq 1:32$ in the diagnosis of legionellosis would be 93%.

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TABLE 1. Distribution of serum MA antibody titers

Titer	Serogroup 1		Serogroup 2		Serogroup 3		Serogroup 4	
	No.	%	No.	%	No.	%	No.	%
≤ 4	125	24.2	157	30.4	58	11.2	103	19.9
8	302	54.8	298	57.6	280	54.2	298	57.6
16	82	15.9	58	11.2	161	31.1	105	20.3
32	8	1.6	2	0.4	15	2.9	11	2.1
≥ 64	0		2	0.4	3	0.6	0	

TABLE 2. Prevalence of serum MA antibody titers of $\geq 1:32$

Feature	Frequency ^a
Age (yr)	
0-19	7/107 (6.5)
20-39	16/170 (9.4)
40-59	12/139 (8.6)
≥ 60	3/101 (3.0)
Sex	
Male	15/249 (6.0)
Female	23/268 (8.6)

^a Percentages are given within parentheses.

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