

## Indirect Immunofluorescence Antibodies to *Legionella pneumophila*: Frequency in a Rural Community

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The background prevalence of indirect immunofluorescence antibody to *Legionella pneumophila* in a rural community was determined by testing sera from 517 volunteers. The upper limit of normal antibody titer was found to be 1:64 with *L. pneumophila* serogroup 1 (Philadelphia 1) used as antigen. High titers ( $\geq 1:128$ ) were found in 13.2% of the sera and occurred with similar frequencies in males and females. In individuals 40 years of age and older, however, high titers were 2.7 times as prevalent in males as females.

The indirect immunofluorescence (IF) test has proved valuable in diagnosing legionellosis (9). With increasing availability and physician awareness of this serological tool, *Legionella pneumophila* infections are being recognized more frequently (2). Determination of the background prevalence of IF antibody to *L. pneumophila* is crucial for a correct interpretation of serological results. Moreover, such information adds to our knowledge of the frequency of *L. pneumophila* infection in different epidemiological settings.

To date, prevalence studies of *L. pneumophila* antibodies have focused on urban populations or have been done in association with investigations of clusters and epidemics of legionellosis (1, 4-6, 8-10, 12). Comparable studies of rural populations have not been reported. We report here the distribution of IF antibody in the population of a rural town in Iowa.

### MATERIALS AND METHODS

**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 old; 249 were male and 268 were female. Eighty-nine percent were residents, and 78% worked in the community. None of the volunteers was acutely ill. Conversations with two local physicians and 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 and Co., Rutherford, N.J.)

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and allowed to clot overnight at 5°C. Tubes were centrifuged, and the sera were collected and stored in 1-ml aliquots at -60°C in screw-capped glass vials. Sera were assayed within 4 months of storage.

**Indirect IF test.** The antigen used was *L. pneumophila* serogroup 1 (Philadelphia 1) supplied by the Center for Disease Control, Atlanta, Ga.

Heat- or diethyl ether-killed *L. pneumophila* suspensions were applied to acetone-resistant, 12-well slides (Cel-Line Associated, Inc., Minolta, N.J.) with a pipette, and excess fluid was removed. Slides were air dried for 30 min at room temperature, acetone-fixed for 15 min, air dried for an additional 15 min, and stored at -20°C. Antigen slides were used within 5 days of preparation.

The test was performed as described by Wilkinson et al. (13). Serum was diluted 1:16 in 3% normal yolk sac solution, and subsequent doubling dilutions were made in phosphate-buffered saline, pH 7.6. The indirect IF conjugate was a fluorescein isothiocyanate-labeled rabbit anti-human immunoglobulin obtained from the Center for Disease Control or obtained commercially (Bionetics Laboratory Products, Kensington, Md.). Both conjugates (the latter diluted 1:2 in phosphate-buffered saline) produced comparable results in our hands. Serum was initially tested at dilutions of 1:16 through 1:512. Specimens with titers equal to or greater than 1:512 were further diluted to obtain a final titer.

Control sera with titers against *L. pneumophila* serogroup 1 of 1:16 and 1:512 were routinely included when examining test sera. Results of the test were considered invalid if the titers obtained with the control sera varied more than twofold from their established values.

The IF titer was the highest dilution giving a 1+ fluorescence with antigen. Comparable titers were obtained with heat- or diethyl ether-killed *L. pneumophila*. In instances in which titers obtained with the two antigen preparations differed by twofold, the lower titer was used.

## RESULTS

The distribution of serum IF antibody titers against *L. pneumophila* serogroup 1 in the population is shown in Table 1. Antibodies were found in titers  $\geq 1:64$  in 32%,  $\geq 1:128$  in 13.2%, and  $\geq 1:256$  in 4.5% of sera examined. Therefore, the upper limits of normal titer by the IF test in this population would be 1:64, since 85% of the specimens tested did not exceed this titer (7).

As shown in Table 2, the prevalences of high antibody titers ( $\geq 1:128$ ) in males (14.9%) and females (11.6%) were not significantly different. In individuals 40 years of age and older, however, the frequency in males (17.9%) was significantly greater than that of females (6.7%), for a male-to-female ratio of 2.7:1.0. In addition, the frequency for females less than 40 years old (16.4%) was significantly greater than that of females 40 years of age and older (6.7%).

The youngest and oldest individuals with antibody titers  $\geq 1:128$  were an 8-year-old girl and an 84-year-old woman. The youngest and oldest males with antibody titers  $\geq 1:128$  were 10 and 68 years of age, respectively.

## DISCUSSION

The prevalence of IF antibodies to *L. pneumophila* has been determined with sera from several epidemiological settings and has been found to vary widely. In urban populations the

TABLE 1. Frequency of IF antibody titers

Titer	Serogroup 1	
	No.	%
$\leq 1:32$	352	68.0
1:64	97	18.8
1:128	45	8.7
1:256	20	3.9
1:512	2	0.4
$\geq 1:1024$	1	0.2

TABLE 2. Age and sex frequencies of IF antibody titers  $\geq 1:128^a$ 

Age (yr)	No. $\geq 1:128$ /total (%)	
	Males	Females
<40	18/143 (12.6)	22/134 (16.4)
$\geq 40$	19/106 (17.9)	9/134 (6.7)
Total	37/249 (14.9)	31/268 (11.6)

<sup>a</sup> The following statistical analysis were made by chi-square analysis (Yates modification):  $P < 0.025$  for females <40 and females  $\geq 40$ ;  $P < 0.025$  for males  $\geq 40$  and females  $\geq 40$ ;  $P < 0.10$  for all males and all females.

frequency of IF antibody titers  $\geq 1:128$  has been very low, only 0.1 to 1.3% of samples examined (8, 12). In contrast, in settings associated with epidemics and large case clusters of legionellosis, IF titers  $\geq 1:128$  occur more frequently and have been found in 4 to 19% of sera (1, 4-6, 9, 10). The 13.2% prevalence of IF titers  $\geq 1:128$  in the present rural setting is surprisingly similar to rates in epidemics and clusters and may indicate past epidemic infection with *L. pneumophila* or a closely related microbial species. An equally plausible explanation, however, would be that the community prevalence is not different than the background prevalence for Iowa. We feel that the latter explanation is more likely the case, based on results of IF tests on 1,049 consecutive sera from patients with acute respiratory disease performed at the University of Iowa Hygienic Laboratory between 1979 and 1980. The prevalence of IF titers  $\geq 1:128$  in this population was 13.9%, quite similar to that of the rural population (unpublished data).

Serological evidence of infection with *L. pneumophila* was found in all age groups within the population, and the overall prevalences of infection in males and females were similar. The frequency of IF titers  $\geq 1:128$  in men over the age of 40 was 2.7 times that seen in women of comparable age, however. This observation is consistent with the male-to-female ratio seen in cases of *L. pneumophila* pneumonia (2, 11). Interestingly, females over the age of 40 years had the lowest frequency of IF titers  $\geq 1:128$ . Whether women in this age group were less frequently exposed to *L. pneumophila* or whether they had fewer risk factors cannot be said with certainty.

In a previous study of IF antibodies in 1,143 sera, the prevalence of seropositivity did not vary with age or sex (12). This study differed from the present study in several respects, however. First, the prevalence of titers  $\geq 1:128$  was considerably lower (1.3%) than in the present study. Second, the sera were obtained from several urban loci, rather than from a single rural location. Third, sera were obtained only from individuals over the age of 46 years, excluding younger individuals. Any one of these factors may have led to differing results.

*L. pneumophila* resides in soil, soil animals, and water (3) and should be widespread in rural areas and agricultural states. In such an environment and provided that appropriate modes of transmission or amplification of infection such as water containing heat-exchange devices were available, the *L. pneumophila* infection frequency might well be increased. Consistent with this hypothesis are (i) the high IF seropositivity

rate in this rural population, (ii) the high IF seropositivity rate in Iowa's acute respiratory sera, and (iii) the frequency of *L. pneumophila* infections among atypical pneumonias in Iowa (11) which appears to be two to four times greater than that reported by others (9). Confirmation of these seroepidemiological findings in other rural and agricultural settings is necessary to lend further support to this hypothesis.

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