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*PREVALENCE OF ANTIBODIES TO LEGIONNAIRES' DISEASE.
A SEROEPIDEMIOLOGIC SURVEY OF MICHIGAN RESIDENTS
USING THE HEMAGGLUTINATION TEST.*

presented by

DANIEL C. EDSON

has been accepted towards fulfillment
of the requirements for

M.S. degree in CLINICAL LABORATORY SCIENCE
DEPARTMENT OF PATHOLOGY

Bertina B. Wentworth Ph.D.

Major professor

Date *February 19, 1979*



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USING THE HEMAGGLUTINATION TEST.

By

Daniel C. Edson

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ABSTRACT

PREVALENCE OF ANTIBODIES TO LEGIONNAIRES' DISEASE.
A SEROEPIDEMIOLOGIC SURVEY OF MICHIGAN RESIDENTS
USING THE HEMAGGLUTINATION TEST.

By

Daniel C. Edson

Comparison of the indirect fluorescent antibody (FA) test with a microhemagglutination technique showed these methods to give approximately the same results for demonstration of antibodies in human sera to Legionnaires' disease antigen. Since the hemagglutination technique was simpler, more rapid and less expensive to perform than the indirect FA, the former was used to determine the prevalence of antibody in 1,200 sera from apparently healthy, non-hospitalized Michigan residents. Prevalence was analyzed by age, sex, geographic location (county of residence) and season of the year. There was a significant difference in prevalence between seasons: 91 of 600 sera (15.2 per cent) from February-March, 1978, had a titer equal to or greater than 1:16 contrasted with 179 of 600 sera (29.8 per cent) from August-September, 1978. This difference was independent of age and sex. There was no significant difference by geographic location, sex or age, except for decreased prevalence for ages 60 and older.

For Golo

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Chance favors the prepared mind.

Pasteur

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INTRODUCTION

Since the Philadelphia outbreak in 1976, sporadic cases and minor outbreaks of Legionnaires' disease have been identified both in the United States and other countries. Although the etiologic agent has been isolated from water at four sites where outbreaks have occurred (7, 8, 9, 20), the reservoir in nature and means of dissemination of this organism are not yet understood, and much remains to be learned about the distribution of this agent in the environment. The prevalence of antibody in the general population would be an indication of the risk of exposure and suggest the frequency of mild or asymptomatic infections due to this bacterium.

This thesis describes a seroepidemiologic survey of antibody to Legionnaires' disease bacterium in Michigan. The study was designed to determine the prevalence of antibody in a non-hospitalized population, according to age, sex, geographic location (county of residence) and season of the year (winter versus summer).

A microhemagglutination technique was developed for use in this study and was shown to give comparable results to the indirect fluorescent antibody (FA) test.

LITERATURE REVIEW

The Philadelphia outbreak

A highly publicized outbreak of pneumonia occurred during the convention of the Pennsylvania chapter of the American Legion in Philadelphia in July, 1976. The disease affected primarily Legionnaires who attended functions and/or lived at the Bellevue-Stratford Hotel during the convention period of July 21-24. Hence, the name "Legionnaires' disease".

A case was considered Legionnaires' disease if it met clinical and epidemiologic criteria. The clinical criteria required that a person have onset between July 1 and August 18, 1976, of an illness characterized by cough and fever (temperature of 38.9 C or higher) or any fever and chest x-ray evidence of pneumonia. To meet the epidemiologic criteria, a patient either had to have attended the American Legion convention held July 21-24, 1976, in Philadelphia or had to have entered the Bellevue-Stratford Hotel between July 1 and the onset of illness (19). There were 182 confirmed cases, of which 29 (16 per cent) were fatal.

Of the 182 cases meeting the clinical and epidemiologic definition of Legionnaires' disease, 142 were males. Patients ranged in age from three to 82 years; 75 per cent were 40 to 69 years old, with a mean of 54.7 years. One hundred and forty-nine had attended the American

Legion convention. One case was in a hotel employee, an air conditioner repairman. The other 32 cases had been inside the hotel but were not associated with the American Legion convention.

About the same time, another 39 persons who had not attended the Legion convention nor entered the hotel were found to have a similar illness. These patients had been on the sidewalk and/or street adjacent to the hotel (Broad Street). Hence, the name "Broad Street pneumonia". A person was considered to have Broad Street pneumonia if he met the clinical but not the epidemiologic criteria for Legionnaires' disease but had been within one block of the Bellevue-Stratford Hotel between July 1 and onset of illness (19). Of the 39 cases meeting this definition, five (13 per cent) were fatal.

For the Legionnaires' disease cases, the earliest symptoms were malaise, muscle aches, headache and a nonproductive cough. Within less than a day there was a rapidly rising fever associated with shaking chills. Two or three days after onset of illness, the temperature had usually risen to 38.9 to 40.6 C, and examination of the chest disclosed some rales, without evidence of consolidation. Radiographs of the chest revealed patchy, interstitial infiltrates or areas of consolidation which usually progressed to more widespread consolidation.

In most cases the illness progressed over two or three days. Cough commonly became productive during the course of the illness but was rarely purulent. In those patients who recovered, radiographic evidence of improvement lagged behind clinical resolution. Outcome was most favorable in patients treated with tetracycline or erythromycin. Characteristic pathological findings were limited to the lungs, in which pneumonia and acute diffuse alveolar damage were seen. Many

bacilli were consistently seen in affected alveoli with a silver-impregnation stain (12), but other stains commonly used did not demonstrate these bacilli.

Further studies of the clinical, epidemiologic and laboratory results indicated that Broad Street pneumonia and Legionnaires' disease were in fact the same.

Recovery of the causative organism

Extensive laboratory methods failed initially to reveal toxins, bacteria, fungi, chlamydiae, rickettsiae or viruses which could have caused the outbreak. Subsequent work resulted in the isolation of a bacterium, not previously recognized as an agent of human disease, in guinea pigs inoculated with lung tissues from four fatal cases (24). The etiologic role of this organism was demonstrated by indirect fluorescent antibody tests on serum specimens from surviving patients. This bacterium, a bacillus, was found to be Gram-negative and not acid fast. The organism did not grow on trypticase soy, blood agar or in thioglycollate broth.

The first direct in vitro isolation of the organism was obtained from a pleural fluid by M. Dumoff (5). He used GC base chocolate agar, containing 1 per cent hemoglobin and 1 per cent Isovitalex.

A link to previous illness

Testing of stored sera from the serum bank at the Center for Disease Control showed that the outbreak of Legionnaires' disease

was similar in many respects to two previous large outbreaks of febrile disease, one in 1965 in the District of Columbia (3) and the other in 1968 at Pontiac, Michigan (4).

The District of Columbia outbreak involved patients in a large psychiatric hospital in which there were 81 cases and 12 deaths. The clinical picture was similar to that observed in the 1976 Pennsylvania outbreak.

The Pontiac episode of acute febrile illness involved personnel and visitors in an office of the county health department in July, 1968. There were 144 cases and no deaths. Typically, there was an acute onset of fever, with chills, myalgia and minor respiratory symptoms. Pneumonia was not seen.

Case profiles

Sparked by these findings, intensive investigations have been ongoing for nearly two years. As of November 10, 1978, 1054 cases of Legionnaires' disease have been confirmed in the United States (10). At least 40 cases have been reported to date from other countries (6, 11, 22, 23) with illnesses originating in Britain, Spain, Italy, Germany, Austria and Australia.

Diagnostic criteria for Legionnaires' disease include: 1) sero-conversion, a four-fold increase in serum antibody titer to a minimum titer of 64; 2) seropositive, a single serum antibody titer of 128 or greater; 3) direct in vitro isolation of the organism; and 4) staining of the organism in tissue by direct fluorescent antibody technique.

Sporadic cases of Legionnaires' disease constitute 496 of the total 1054 United States cases (10). The earliest case had onset in May, 1973. These 496 cases have occurred in 43 states and the District of Columbia. The majority of sporadic cases have been in eastern and midwestern states with Michigan reporting the greatest number - 55.

In 437 sporadic cases where the sex of the patient was reported, 309 were men and 128 were women. The youngest patient was a two-year-old boy; the oldest patient was an 84-year-old man. The median age for males was 54 years, for females 56 years. Death directly attributable to Legionnaires' disease has occurred in 90 (19 per cent) of the sporadic cases in which the outcome was known (10).

A total of 558 confirmed outbreak-associated cases have been reported by the Center for Disease Control (10). These outbreaks have occurred in Ohio, Vermont, Tennessee, California, Georgia, New York, Michigan, Indiana, Texas, Pennsylvania and the District of Columbia.

Males represented 412 of these cases, females 146. There were 70 deaths among these 558 cases, representing 14 per cent of cases presenting with pneumonia.

The emerging clinical picture of Legionnaires' disease suggests that this disease may present as pneumonia severe enough to cause respiratory failure and death in a previously healthy person (1), or as only an acute febrile illness without pneumonia (20). The variations between these extremes still need to be described. Pathology studies on lung tissue indicate that the macroscopic lesions are compatible with an inhalation etiology and with a bronchiolar origin of the inflammatory process (33). It does appear that underlying disease is a significant risk factor. Estimates of

incidence at this point are at best only guesses.

The bacterium

The nature of the macroscopic lesions from lung tissue coupled with epidemiologic studies from outbreaks of Legionnaires' disease suggest that the causative organism may be quite common in the environment and that the mode of spread is probably air borne. As a result of investigations of outbreaks in Pontiac, Michigan, Memphis, Tennessee, Bloomington, Indiana and Atlanta, Georgia, the Legionnaires' disease bacterium has been isolated from water from two cooling towers, three evaporative condensers and a creek (7, 8, 9, 20).

Thirty clinical isolates of the Legionnaires' disease bacterium have been reported as of November 10, 1978 (2). The organism is a Gram-negative, weakly oxidase-positive, catalase-positive, fastidious bacterium that has a narrow optimal pH and temperature range. It will not grow under anaerobic conditions. The Legionnaires' disease bacterium produces a soluble brown pigment, utilizes starch and liquifies gelatin. It does not reduce nitrates, utilize carbohydrates or attack urea. It does not appear to possess decarboxylases for either lysine or ornithine, or an arginine dehydrolase. The organism will grow only on a medium which utilizes a peptone base (GC agar base) and not on those media which use trypticase soy base suggesting a nutritional requirement for some particular amino acid.

Cellular fatty acid composition studies by gas-liquid chromatography and mass spectrometry indicate the organism contains large amounts

(81 to 90 per cent) of branched-chain acids (25). This appears to be a unique fatty acid profile.

The simple and complex lipid composition of the Legionnaires' disease bacterium has been studied by Finnerty et al. (18). The major phospholipids were, in decreasing order of concentration, phosphatidylcholine, phosphatidylethanolamine, cardiolipin, phosphatidylmonomethylethanolamine, and phosphatidylglycerol. The ten isolates studied averaged 96 micromoles of total phospholipid phosphorus per gram dry cell weight.

The fatty acid composition of the total cellular phospholipids was 99 per cent branched chain fatty acids that consisted, in decreasing order of concentration, of iso-C16:0, anteiso-C15:0, anteiso-C17:0, iso-C14:0 and iso-C16:1.

The simple cellular lipids consisted of free fatty acid, ubiquinone, triglyceride, and an unknown fluorescent component. The fatty acid composition of the total cellular neutral lipids ranged from 52-87 per cent branched chain fatty acids; iso-C16:0 was the major component. The normal saturated and monosaturated fatty acids were, in decreasing order of concentration, C16:0, C17:0, C15:0, C14:0, and C16:1.

The phospholipid and fatty acid composition of this organism are unique according to Finnerty et al. (18). Phosphatidylcholine is widely distributed and quantitatively important in animals and higher plants, but is generally absent among the prokaryotic bacteria. The orders Pseudomonadales and Eubacteriales have genera in which several species contain phosphatidylcholine but at significantly

lower concentrations than that found in Legionnaires' disease bacterial isolates. These studies serve to set these isolates apart from the vast majority of prokaryotic bacteria.

A major antigen has been separated and purified from the Knoxville strain of this organism by Wong et al. (18). The antigen contained all the major fatty acids characteristic of the Legionnaires' disease bacterium and appeared to be a lipid-protein-carbohydrate complex. SDS acrylamide-gel electrophoresis resolved the antigen into four protein bands (molecular weights 720,000, 390,000, 270,000, and 90,000) and one glycoprotein band (molecular weight 50,000). The purified antigen was practically non-toxic to animals. It induced little or no Schwartzman reaction in rabbits and was well tolerated by young mice in both intraperitoneal and subcutaneous injection.

Although the Legionnaires' disease bacterium is a Gram-negative organism, its endotoxicity appears to be different from the classical endotoxicity generally associated with Gram-negative bacteria. Analysis by gas chromatography of Knoxville strain cells and their cellular fractions from phenol extraction by Wong et al. (31) indicated that the organism did not contain the fatty acids characteristic of lipid A of classical endotoxin.

Biologically, the Knoxville strain cells and cell extracts were highly reactive in the limulus lysate test, causing gelation of lysates of Limulus polyphemus amebocytes, in vitro, indicative of the presence of endotoxin-like substance. The same cell preparations, however, contained little pyrogenic activities in rabbits. Other in vivo biological tests, including Schwartzman reaction, actinomycin D

potentiation, weight gain in mice, and polymixin B inactivation also indicated that laboratory grown organisms contained little endotoxicity (31).

Transmission electron microscopy of the Legionnaires' disease bacterium revealed electron lucent nucleoids interspersed among areas of well-defined ribosomes; cleanly circumscribed cytoplasmic vacuoles; and a double envelope enclosure, each portion of which consisted of a triple-layered "unit" membrane, approximately 75 Å wide (13). No flagella were seen nor were unique features found that would aid in the ultrastructural differentiation of this organism from other small Gram-negative bacilli (26).

The Legionnaires' disease bacterium deoxyribonucleic acid (DNA) has a guanine plus cytosine (G+C) content of 39 per cent and a genome mass of approximately 2.5×10^9 daltons (2). DNA's from seventeen strains examined by Brenner et al. (2) as of November 10, 1978, were 80 per cent or more related; therefore all of these strains belong to the same species.

Morphological and biochemical data comparison, G+C content, and DNA hybridization have been used to determine whether the Legionnaires' disease bacterium is a member of a previously described genus or represents an entirely new genus. The organism is genetically unrelated to all organism tested at both the species and genus levels. Brenner et al. (2) have concluded that the Legionnaires' disease bacterium appears to be a previously undescribed genus and have proposed the scientific name Legionella pneumophila for this organism.

Four serotypes of this bacterium have been described (16). Serotype 1 consists of most isolates (including the Philadelphia and all Michigan strains); serotype 2 contains the Togus, Maine patient

isolate as well as Atlanta 1 and 2 cooling tower water isolates; serotype 3 is the Bloomington 2 strain recovered from river water; while serotype 4 is the Los Angeles 1 strain, also a patient isolate. It is probable that additional serogroups will be identified as more organisms are isolated, necessitating the use of broad spectrum reagents for maximum antibody detection.

In addition to the indirect fluorescent antibody procedure described by McDade et al. (24) for detection of serum antibody to this organism, a microhemagglutination test and micro-ELISA test have been reported by Farshy et al. (17). Both of these latter two tests are in the experimental stages only and are not being used routinely at the Center for Disease Control due to technical difficulties.

The need for a reliable yet rapid and inexpensive technique for examining large numbers of sera led to the development of a microhemagglutination method described and used in this research.

MATERIALS AND METHODS

Specimens for comparison of hemagglutination technique to indirect fluorescent antibody (FA) test

For the comparative study, 430 sera submitted to the Michigan Department of Public Health for Legionnaires' disease testing were evaluated by both the indirect FA procedure and the hemagglutination technique. These included 377 single and 53 serial serum specimens.

Population selection for seroepidemiologic survey

Sera from 1,200 apparently healthy, non-hospitalized Michigan residents were obtained from specimens submitted to the Michigan Department of Public Health for premarital or preemployment syphilis serology. None of the sera were reactive in the VDRL test. A total of 600 sera were examined for each of two seasons: late winter (February-March, 1978) and late summer (August-September, 1978). The only criterion for inclusion of samples in the study was sufficient volume for testing and sera were assigned to the study until there were 50 females and 50 males represented in each of the following six age groups: 15-19, 20-29, 30-39, 40-49, 50-59 and 60 years or older.

Premarital sera constituted 34.7 per cent of the male sera and 41.7 per cent of the female sera for ages 15 to 59 years of age. The remainder of sera were preemployment specimens submitted by Michigan industries. Only 20 per cent of the sera for 60 years or older ages was premarital and 80 per cent was preemployment.

Indirect FA procedure

A standard indirect FA procedure was employed (14), using as antigen a formalinized cell suspension of Flint No. 1 isolate, (supplied by Dr. Morris Dumoff, Flint, Michigan) grown on enriched chocolate agar (GC base medium with heated sheep blood and vitamin supplement). Sera were tested at initial dilutions of 1:16.

This procedure was standardized initially by comparing results of testing split serum samples with those obtained by the Center for Disease Control (CDC). Control reagents supplied by CDC were tested with each set of unknown sera to maintain proper quality control.

Hemagglutination procedure

The hemagglutination test was similar to that described by Wentworth et al. (30) for the detection of treponemal antibodies. Turkey erythrocytes, sensitized by the bis-diazotized benzidine technique with agar grown cultures of three Michigan isolates (one Detroit and two Flint strains), were used as antigen (Difco Laboratories, Inc., Detroit, Michigan). Test sera were screened at a 1:16 dilution against these sensitized cells and unsensitized control cells and were heated at 56 C for 30 minutes before testing. U-type 96-well

polystyrene microtitration plates (Linbro Scientific, Hamden, Connecticut) were used for these tests. Plates were incubated 2 hr at room temperature ($25\text{ C} \pm 5\text{ C}$). Sera that agglutinated test cells on initial screening were titrated in twofold dilutions with sensitized and control cells. Sera which agglutinated sensitized but not control erythrocytes were considered reactive at the highest dilution showing demonstrable agglutination.

Statistical analysis

The results of hemagglutination tests were tabulated for the two seasons by age, sex and geographic location and analyzed by the chi-square (χ^2) test (34) (with Yates correction when small numbers were used) for significant differences.

RESULTS

Comparison of hemagglutination procedure with indirect FA test

Preliminary investigations showed that testing 1:8 dilutions of sera in the hemagglutination procedure yielded an excess of nonspecific reactions with control cells and further dilutions of these sera were nonreactive with both sensitized and unsensitized cells. Testing at an initial dilution of 1:16 eliminated this problem. The results of hemagglutination tests were compared to the results obtained by the indirect FA technique on sera submitted for Legionnaires' disease testing.

There was no statistically significant difference in the number of sera with titers equal to or greater than 1:16 among the 430 sera examined by indirect FA and the hemagglutination technique, with 94.9 per cent agreement between techniques. Overall, 69 (16 per cent) of these sera were reactive in the indirect FA test and 61 (14.2 per cent) in the hemagglutination test. Seven sera were reactive in the hemagglutination test only, while 15 sera were FA reactive but not reactive with hemagglutination. The majority of these sera (nine) had FA titers of only 1:16.

Among 54 sera showing titers in both tests, 16 had identical titers, 23 agreed within one twofold dilution, seven varied by one

fourfold dilution and eight by more than fourfold. In 16 instances the indirect FA gave the higher titer and in 22 cases the hemagglutination titer was higher.

Serial sera from 21 pneumonia cases showed fourfold or greater rises in titer to at least 1:128 in the indirect FA test, four of which would not have been detected by similar rises in the hemagglutination procedure since only stationary titers were seen with hemagglutination. Two of five single sera from pneumonia cases with indirect FA titers of 1:128 did not have similar hemagglutination titers. One pneumonia case showed low stationary titers in single sera in the indirect FA test but a fourfold rise to 1:256 with hemagglutination.

Although it appeared that hemagglutination may be slightly less sensitive than the indirect FA technique for detecting antibody to this organism, the hemagglutination procedure was used in the prevalence study for practical reasons, since it was considered that a less sensitive technique would yield a minimum estimate of prevalence compared to results with a more sensitive method.

Prevalence of hemagglutination antibody to Legionnaires' disease bacterium

The prevalence of antibody for premarital sera was not different from that for preemployment sera ($p>0.10$); therefore, the results have been combined.

As shown in Table 1, 270 of the 1,200 serum specimens had detectable antibody for an overall prevalence of 22.5 per cent. Examination of

Table 1. Number and per cent of sera with hemagglutination titers equal to or greater than 1:16 to Legionnaires' disease bacterium.

Age in Years	Winter				Summer				Total	
	Males		Females		Males		Females		Combined	
	<u>No.</u>	<u>%</u>	<u>No.</u>	<u>%</u>	<u>No.</u>	<u>%</u>	<u>No.</u>	<u>%</u>	<u>No.</u>	<u>%</u>
15-19	7*	14	13*	26	17*	34	15*	30	52	26
20-29	8	16	10	20	15	30	26	52	59	29.5
30-39	8	16	3	6	16	32	17	34	44	22
40-49	8	16	11	22	9	18	22	44	50	25
50-59	11	22	4	8	14	28	11	22	40	20
60+	2	4	6	12	10	20	7	14	25	12.5
Total	44	14.7	47	15.7	81	27	98	32.7	270	22.5

*Of 50 sera tested

the data revealed a statistically significant difference in prevalence by season ($p < 0.001$) in that 91 of 600 (15.2 per cent) of the sera from the winter group and 179 of 600 (29.8 per cent) of the summer group had detectable antibody. In both winter and summer groups there was a trend toward decreasing prevalence from the fourth through sixth decades, but only prevalence at ages 60 or greater was significantly different from prevalence at other ages ($p < 0.01$).

No trend was found when comparing females and males within age groups even though four female groups did show differences from their corresponding male groups ($0.05 > p > 0.01$). Winter female 30-39 and 50-59 age groups showed less prevalence of antibody than the male groups, while summer female 20-29 and 40-49 age groups showed greater prevalence. These differences may be due to the small number of sera (fifty) in each group.

Geometric mean titers were calculated by age and season (Table 2) for those sera showing reactive results. All but one of the winter groups showed a higher geometric mean titer than that for their corresponding age group from the summer. The single exception (60 years and older) consisted of only eight sera with titers, so the low geometric mean titer (32.0) may be due to a small sample. The overall winter geometric mean titer (50.2) was significantly higher than that of the summer (38.8).

Table 3 shows the distribution of titers obtained in this study. The majority, or 76 per cent, of reactive sera had titers of 1:32 or 1:64, and the overall prevalence of antibody titers equal to or greater than 1:32 was only 19.3 per cent since only 3.2 per cent

Table 2. Geometric mean titers to Legionnaires' disease
by age and season.

Age in Years	Winter	Summer
	geometric <u>mean titer</u>	geometric <u>mean titer</u>
15-19	51.9	38.0
20-29	52.8	49.6
30-39	64.0	35.5
40-49	51.4	35.8
50-59	53.2	34.8
60+	32.0	36.2
Overall	50.2	38.8

Table 3. Antibody titers to Legionnaires' disease by season and sex.

<u>Titer</u>	Winter		Summer		<u>Totals</u>
	<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>	
1:16	1	3	15	19	38
1:32	17	18	42	38	114
1:64	19	20	17	36	92
1:128	6	6	5	4	21
1:256	1	0	3	0	4
1:512	0	0	0	1	1

of sera titered 1:16. There was, therefore, a statistically significant difference between prevalence of sera with titers of 1:32 and 1:64 in winter versus summer samples.

In both the winter and the summer series, 2.2 per cent (13 of 600) of the sera had titers equal to or greater than 1:128. There was no concentration of titers of this magnitude in any age group and no significant difference between the number of males and the number of females with such titers.

DISCUSSION

This study has shown that the hemagglutination procedure compares favorably with the indirect FA technique for detection of antibody to Legionella pneumophila. Because the hemagglutination method is simpler, more rapid and less expensive than the indirect FA procedure, the hemagglutination test is well suited for large seroepidemiologic surveys.

Among those serum samples available to us, premarital and preemployment sera were chosen for this study as most representative of the general population. Since there was no difference in prevalence between premarital and preemployment sera, the type of employment was not investigated further. The sera came from 50 of the 68 counties in the lower peninsula of Michigan and were distributed essentially in accord with the population, as shown by the fact that 43.5 per cent of the 1,200 serum samples came from three of the four largest counties in the state whose combined population represents 43.2 per cent of the state population. The remaining specimens were distributed among the smaller counties approximately by county population. Prevalence of antibody for the larger urban counties was not significantly different from that for the smaller, more rural counties. There are no data to show whether this sample does or does not represent the general population of the state, but there is no reason to assume

that those individuals applying for employment or a marriage license are different from the population at large.

Although it appears that acquisition of antibody which reacts with antigen prepared from the Legionnaires' disease bacterium is associated with pneumonia due to this organism, there is as yet insufficient evidence to determine fully the significance of antibody in single serum specimens from asymptomatic individuals.

It is possible that such antibody may be due to exposure to cross-reacting antigens as well as exposure to this bacterium, even though no cross reactions between Legionella pneumophila and other antigens have been conclusively demonstrated as yet (16). Extensive studies will be needed to establish the antigenic specificity of the antigens of this organism, and to determine whether any cross-reactivity can influence antibody prevalence.

Since it has been reported that 50 per cent of patients from the Philadelphia outbreak in 1976 had titers less than 1:128 by six months after the outbreak (28), lower titers may indicate past infections, and it seems reasonable to assume, until there is data to indicate otherwise, that here, as with other diseases such is the case.

The 22.5 per cent overall prevalence of antibody in the population studied appears to further substantiate the hypothesis that this organism is widespread in nature. Although sporadic cases identified so far in Michigan show a male to female ratio of 1.57 to 1, no increased prevalence of antibody among males was shown in this study.

The seasonal variation in prevalence (winter - 15.2 per cent, summer - 29.8 per cent) may be due to the severe winter climate in

Michigan if exposure to the organism is water and/or soil related. The higher geometric mean titer (50.2) associated with lower prevalence for the winter months may indicate that exposure during this "flu and pneumonia" season is more likely to result in respiratory disease and that these sera may have been drawn before post-infection titers have declined. Onset of warm summer temperatures may result in a wider dissemination of this agent, with resulting higher prevalence of antibody. By late summer, levels of antibody due to this exposure may have declined, or exposure during this season may not give rise to respiratory illness with enhanced antibody response, thus a lower geometric mean titer (38.8) for the August-September sera.

A prevalence of this magnitude in an apparently healthy population makes it clear that exposure to this agent is not limited to epidemic situations and suggests that exposure is relatively widespread among the general population. This exposure leads to some frank disease, as shown by detection of sporadic cases.

The relatively constant prevalence of antibody in adults without increasing prevalence by age and with a slight decrease in the older age groups is similar to the prevalence curve for varicella-zoster virus (29), suggesting the possibility that exposure to Legionella pneumophila also occurs primarily in an age group younger than those examined in this study.

Further studies are needed to determine the specificity of antibody being detected as well as antibody prevalence in selected populations and in various geographic locations. Specificity of antibody detected by hemagglutination would best be determined by cross-absorption

studies with antigens for which cross reactivity has been suggested: Mycoplasma pneumoniae (21), Chlamydia psittaci (27), and Pseudomonas fluorescens (15).

To test the hypothesis that primary exposure may occur at ages less than 15 years it would be necessary to examine a significant number of sera from apparently healthy children. Both a late winter and a late summer group would be desirable to effectively compare these results with those obtained in the 1,200 sera study. Specimens of this nature might most easily be obtained from grade school children participating in an experimental vaccine trial or other similar project through a Health Maintenance Organization.

Geographic analysis of prevalence in the United States would best be accomplished by a state-by-state investigation on a population similar to that studied in this research. An extended study within the state of Michigan to include the upper peninsula could be performed. One potential survey would involve dividing the state into three zones (by county): upper peninsula, northern lower peninsula and southern lower peninsula. A predetermined number of sera could be examined for presence of antibody and the results tabulated by age and sex. An alternative to this would be to repeat the entire 1978 study in 1979 with inclusion of sera from the upper peninsula. County of residence could then be examined in a similar manner to the 1978 survey.

Studies of this type could yield clues concerning the reservoir and dissemination of this organism.

Although much remains to be discovered concerning the epidemiology of this newly recognized disease, it is now apparent that it occurs in both epidemic and sporadic forms, as a severe pneumonia or milder respiratory illness, and that infection may be quite common, since 22.5 per cent of the persons over the age of 15 appear to have serum antibody.

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