

A prevalence study of antibodies to *Legionella* spp. in geriatric institutions

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(Received 12 May 1983; accepted 21 June 1983)

SUMMARY

The prevalence of seropositivity to *Legionella* spp. was studied in inmates and staff members of two geriatric hospitals (Rahel and Sinai); attendants to a geriatric Day Club; and 26 old people with entirely independent households. The sera were examined by indirect immunofluorescence with antigens of six *Legionella* species (*L. pneumophila*, *L. longbeachae*, *L. micdadei*, *L. gormanii*, *L. dumoffii* and *L. bozemanii*). A titre of ≥ 256 to at least one antigen was obtained in 30.5% of patients and 35.7% of staff members from Rahel; 12.1% of patients and 17.2% of staff members from Sinai; 9.1% of attendants to the Day Club; and none of 26 old people with their own households. All the positive patients from Rahel reacted with *L. pneumophila*, but only four of seven of the positive patients from Sinai did so; none of the seropositive subjects from the Day Club reacted with this agent.

The results indicate intramural exposure to agents of legionellosis, at least for the Rahel Hospital.

INTRODUCTION

Only few and sporadic cases of Legionnaires' Disease have so far been reported from Israel (Berman, Loon & Rubinstein, 1979; Aderka *et al.* 1982). One case was probably hospital acquired (Isakov *et al.* 1982); childhood legionellosis has also been evidenced (Mundel *et al.* 1983). A high incidence of serological reactions to *Legionella pneumophila* has been demonstrated among chronic haemodialysis patients from three hospitals (Boldur *et al.* 1982). We present here a prevalence study of seropositivity to six *Legionella* species among patients and staff members of two geriatric hospitals; attendants of a Day Club for the aged; and old people with independent households. Acute and chronic lower respiratory tract infections are not infrequent among the elderly, and particularly among patients in the geriatric hospitals, but an exact etiologic diagnosis is usually not obtained, and consequently the choice of therapeutic agents is usually not rational.

Table 1. *The study population*

	Rahel Hospital	Sinai Hospital	Day Club	Own household
No. examined (aged persons)	59	58	44	26
Males/Females	11/48	20/38	1/43	10/16
Age (range in years)	65-92	60-92	49-85	60-80
Age (median) in years	80	79	69	66
Staff members examined	14	30	—	—

MATERIALS AND METHODS

The study population

The two hospitals and the Day Club are located a few miles from each other in the town of Ramle, about 12 miles east of Tel-Aviv. The persons with independent households all lived at short distances from the hospitals (not more than four miles). The hospitals were located in old-fashioned two-storyed houses with small gardens. The houses had no air-conditioning. Due to repair work of various kinds during the last two years, soil-excavations were frequently performed. A general inspection showed that hygienic conditions in Sinai were less than satisfactory, whereas in Rahel they were found tolerable, but exact grading was not thought practicable.

Table 1 gives a survey of the 231 individuals investigated. The independent elderly were younger than the patients in the geriatric hospitals. Both the institutionalized and the independent elderly were from the lower or the middle socio-economic classes of the population. Among the inmates in the hospitals and the Day Club, the females outnumbered males by far.

Laboratory methods

Usually only one serum sample was obtained from each person. This was stored at -20°C until examined by the indirect immunofluorescence assay (IFA). Antigens and positive antisera for standardization were obtained from The Center of Disease Control (CDC), Atlanta, Ga., and represented *L. pneumophila*, gr. 1-4 (monovalent and pool); *L. pneumophila*, gr. 5-6 (pool); *L. longbeachae*, gr. 1-2 (pool); and serogroup 1 of *L. micdadei*, *L. gormanii*; *L. dumoffii*; and *L. bozemanii*. IFA was examined according to the technique of the Center of Disease Control (1981), with the exception that the first screening was performed with a commercial (Institute Pasteur, Paris) fluorescein isothiocyanate (FITC) - antihuman gamma-globulin (Ig), and those positive to ≥ 256 were re-examined after absorption with antigen of *Escherichia coli* 013:K92:H4 (Wilkinson *et al.* 1979). With our material, this procedure eliminated or markedly weakened the reactions of about 8% of the sera; these reactions possibly were non-specific. Sera positive to ≥ 256 with fluorescent rabbit anti-Ig were further examined for IgG- and IgM-type antibodies with FITC-antihuman IgG (anti-gamma) and antihuman IgM (anti-mu) (Wellcome Reagents, Ltd). Sera positive for IgM were absorbed on a column of polymerized human IgG (Merlini *et al.* 1979) in order to avoid false reactions due to rheumatoid factors. Only results refractory to this treatment will be reported.

Table 2. Seropositivity to *Legionella* species (≥ 256) in the study groups

	Number	Positive	(%)
Rahel Hospital			
Inmates	59	18	(30.5)
Staff	14	5	(35.7)
Sinai Hospital			
Inmates	58	7	(12.1)
Staff	30	5	(16.7)
Day Club	44	4	(9.1)
Independent aged	26	0	—

The incidence of seropositivity among inmates from Rahel was significantly higher than among patients from Sinai, and among Day Club members ($P < 0.02$, Chi square test) as well as among independent elderly ($P < 0.005$; Fisher's exact test). The incidence among staff members from the two hospitals was not significantly different ($P > 0.05$ by either test). Likewise, the differences between the incidences among Day Club members and independent elderly, and among inmates from Sinai and Day Club members or independent elderly were not significant ($P > 0.05$ by Fisher's exact test).

Table 3. Distribution of positive reactions according to *Legionella* species and serotitre

	No. positive	<i>L. pneumophila</i>				<i>L. micdadei</i>		<i>L. gormanii</i>
		256	512	1024	2048	256	512	512
Rahel Hospital								
Patients	18	9	8	1	—	—	1 ^a	—
Staff	5	1	1	3	—	—	—	—
Sinai Hospital								
Patients	7	3	1	—	—	2	1	—
Staff	5	—	1	3	1	—	—	—
Day Club members	4	—	—	—	—	1	2	1
Independent households	0	—	—	—	—	—	—	—

^a Positive also with *L. pneumophila*.

RESULTS

The prevalence of seropositivity (≥ 256) to *Legionella* species is shown in Table 2. The highest incidence of seropositivity was found among patients (30.5%) and staff members (35.7%) of Rahel. The incidence in Sinai was significantly lower for patients (12.1%) and non-significantly lower for staff members (16.7%); there was an incidence of 9.1% among members of the Day Club, significantly lower than among the patients of Rahel, but not significantly lower than among the patients of Sinai; none of the elderly with independent households were antibody positive to *Legionella* species.

An analysis of the positive results potentiated further the differences between the groups (Table 3). All seropositive subjects from Rahel reacted to *L. pneumophila*, and one of them in addition to *L. micdadei*, whereas only four of the seven positive patients from Sinai, and none of the four positive Club members reacted to *L. pneumophila*. Only one positive reaction to *L. gormanii*, and none to *L. longbeachae*, *L. bozemanii*, and *L. dumoffii*, was observed. Of the 32 positive reactions to *L.*

pneumophila listed in Table 3, three were monovalent (serogr. 1, 2 and 4); 11 reacted only with serogroups 5 and 6 (pool); 11 reacted with all monovalent antigens of serogroups 1-4; and seven reacted with all serogroups 1-6.

One of the patients from Rahel showed a titre of 512-1024 to serogroups 1-4 with anti-Ig and anti-IgG and a titre of 256 with anti-IgM. This patient suffered from chronic bronchitis. She was re-examined 8 months later, when she had a titre of 256 with anti-IgG and 64 with anti-IgM. Another patient had a titre of 256 of anti-IgM to *L. micdadei* in addition to positive IgG reactions to *L. pneumophila*, but she passed away before a follow-up sample was obtained. Other IgM reactions disappeared after adsorption of the serum on a column of polymerized human IgG (Merlini *et al.* 1979) and were probably due to rheumatoid factors.

DISCUSSION

The purpose of this study was to determine whether the inmates of some geriatric institutes experience excess exposure to *Legionella* spp. compared with the outside population. In a study of this kind the diagnostic specificity of the reaction used must be high. We have therefore chosen a relatively high cut-off limit for seropositivity (reciprocal titre ≥ 256). Titres of 64 have been observed at a relatively high frequency in a previous study of healthy controls in our area, even in young people (Boldur *et al.* 1982). Studies in some other geographical areas have also shown a high background prevalence of low-titre (≥ 64 or even ≥ 128) IFA reactions to *L. pneumophila* in healthy population groups (Bettelheim, Metcalfe & Sillars, 1982). These background reactions seem to be particularly frequent in epidemic areas (Storch *et al.* 1979).

A serological titre of ≥ 256 is usually accepted as presumptive for a present or past infection with *Legionella* (Wilkinson *et al.* 1979). The results indicate, therefore, an institutional exposure among patients, particularly with *L. pneumophila* (30%) at the Rahel Hospital. Supporting the thesis of institutional distribution is the high prevalence of reactions among staff members of this hospital. In the Sinai Hospital, only four out of 59 patients (7%) reacted with *L. pneumophila* antigen, but three were positive to *L. micdadei*, and among permanent attendants to the Day Club only four reactions (9%) to *L. micdadei* and *L. gormanii* were observed and these reactions may not have been acquired in the institution.

Previous studies have demonstrated rises in antibody titres during convalescence of legionellosis, that were specific for a single serogroup antigen, or against antigens common in multiple serogroup strains (Wilkinson, Fikes & Cruce, 1979; McKinney *et al.* 1980). If reactions to serogroups 5 and 6 of *L. pneumophila*, which were available only in pool, are counted among the monospecific, we have observed 14 out of 32 (44%) reactions to specific antigens and 18 (56%) to common antigen (s). It has been proposed, but not proven, that the common antigen reactions may be secondary reactions on repeat exposure to a different serogroup strain (McKinney *et al.* 1980).

Many cases of pneumonia in the elderly are not ascribed to a specific aetiology, and the possibility that some of them are due to legionella infection has previously been stressed (Hjelmes, 1980). Individuals over 60 are more susceptible to this infection (Tsai *et al.* 1979) and mortality increases with advanced age (Center for

Disease Control, 1978). It should also be kept in mind that legionellosis is a multisystemic disease with numerous extrathoracic manifestations (Blackmon *et al.* 1981). It is therefore of great practical importance to establish the prevalence of the infection in geriatric institutions. Our results indicate that exposure to the infectious agent might have occurred inside the institution, at least in the case of the Rahel Hospital. *L. pneumophila* has been isolated from hospital shower heads (Cordes *et al.* 1981) and from the water supply of a hospital (Stout *et al.* 1982). Airborne transmission with organisms aerosolized during soil excavations has also been suspected as a source of nosocomial outbreaks (Thacker *et al.* 1978). Though other possibilities were not investigated in this early study, attention should be paid to the frequent digging work in and around the two Ramle Hospitals. Meanwhile, the possibility of legionellosis should be taken into serious consideration when unexplained pneumonia or other infections are treated in inmates of these institutions, and our results should stimulate studies of similar institutions elsewhere.

The medical and administrative staffs of the participating institutions have co-operated in the best way in obtaining the samples for examination and any information required. We also are thankful to Dr Hazel W. Wilkinson, of the Center of Disease Control, Atlanta, Ga., for the kind co-operation in standardization of our IFA reaction. We appreciate the technical assistance of Mrs Soraya Einy.

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Addendum

One and a half years after the serum specimens from Rahel hospital were obtained, we got the opportunity to perform a limited investigation of possible sources of *Legionella* spp. in this institute. Seven shower heads and one water cock were swabbed thoroughly with cotton wool. Water samples (10 ml) were obtained from five watercocks, and 500 ml samples from each of one shower head and two closets. Finally, we examined water from a pail left over in the garden of the institute (about 500 ml). The source of the water was unknown. The institute had no air conditioning system, and the water of the area was chlorinated to a level of 0.1 p.p.m. free chlorine. The swabs were inoculated directly onto CYE agar (Feeley *et al.* 1979) + colistimethate, vancomycin, nystatin (CVN) (Thorpe & Miller, 1980). Water samples were filtered through Millipore pads (0.45 μm), which were incubated on CYE+CVN at 37 °C in a candle jar. Suspected colonies were transferred onto non-selective Mueller-Hinton-IsoVitaleX agar (Feeley *et al.* 1978). Biochemical examination of isolates was carried out as recommended by Blackmon *et al.* (1981) and by the API 20 E system (Bornstein, Marmet & Fleurette, 1981). Suspected organisms were also stained with FITC-conjugated antisera to *L. pneumophila*, serogroups 1-6; *L. bozemanii*; *L. dumoffii*; *L. gormanii*; *L. micdadei*; and *L. longbeachae*, serogroups 1-2 (pool). These sera were a gift from CDC.

Most of the 17 samples yielded mixtures of microorganisms, which could not be identified as legionella, but on the pad used for filtration of the water from the pail, a few colonies appeared after 4 days' incubation. These colonies were suspected as legionella, on morphological grounds, a brown pigment appeared slowly, and when examined under longwave-u.v. they appeared yellow. No growth was obtained on blood agar. Microscopically slender gram negative rods were observed. The bacteria were stained distinctly with FITC-anti *L. pneumophila* gr. 1, but not with any of the other diagnostic sera. The bacteria were positive for

oxidase, catalase, gelatinase, β -lactamase (nitrocefin); they did not attack urea or reduce nitrate; glucose and other carbohydrates were not fermented. Reactions in the API 20E-system were also as described for *L. pneumophila*.

Although this finding proves that *L. pneumophila* exists in the premises of Rahel hospital, we have no evidence that links the isolation from the water sample to possible exposure of the inmates. As far as we know this is the first isolation of *L. pneumophila* from this geographical area.

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