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Seroprevalence of Legionella in Shanxi Province, China

Summary: Using an ELISA with bacterial sonicate antigens, the prevalence of antibodies to the antigens of 15 legionellae was determined in 319 healthy individuals residing in Taiyuan, China (Shanxi Province). Significant antibody levels were detected to 11 antigens. Confirming earlier studies, an association was noted between cigarette smoking and seroreactivity, however, none was observed between occupation, or sex and seroreactivity. When the response to specific antigens or antigenic "groups" was studied, an association with work site was apparent. These data suggested a higher rate of exposure to Philadelphia 1 type antigens at the Bureau of Mining dormitory and a nearby electric power plant, and to E-327F/NY-23 type antigens at the Shanxi Mining College and Shanxi Daily newspaper. Further studies will define the reservoirs and mechanisms of exposure.

Zusammenfassung: *Seroprävalenz von Legionella in der Provinz Shanxi, China.* Die Prävalenz von Antikörpern gegen Antigene von 15 Legionellen wurde mit ELISA unter Verwendung von ultraschall-behandelten Bakterienantigenen bei 319 gesunden Personen bestimmt, die in Taiyuan, China (Provinz Shanxi) wohnen. Gegen 11 Antigene wurden signifikante Antikörperspiegel gefunden. Wie in früheren Studien fand sich eine Assoziation zwischen Zigarettenrauchen und Seroreaktivität; zwischen Berufstätigkeit oder Geschlecht und Seroreaktivität war hingegen keine Beziehung festzustellen. Bei der Analyse der Antikörperantwort auf spezifische Antigene oder Antigen-"Gruppen" war eine eindeutige Beziehung zum Arbeitsplatz erkennbar. Aus diesen Daten ließ sich eine höhere Expositionsrate gegen Philadelphia-Typ-1-Antigene in der Unterkunft des Bergwerkbüros und einem benachbarten Elektrizitätswerk ableiten; im Shanxi Bergwerk-College und bei Mitarbeitern der Zeitung Shanxi Daily erschien die Exposition gegen Antigene vom Typ E-327F/NY23 erhöht. Die Auffindung der Erregerreservoirs und Bestimmung der Expositionsmechanismen ist weiteren Untersuchungen vorbehalten.

Introduction

Legionella pneumophila, an important pulmonary pathogen, may be the second most frequent bacterial cause of community-acquired pneumonia in the U.S.A. (1). Although the first case recognized in China was reported in 1983 (2), and antibody levels against *L. pneumophila* were determined in healthy individuals from Tianjing, Nanjing and Huaiyin (3), the prevalence and distribution of legionella infections in China are still not well-known. Legionellosis rates may be seriously underestimated, since diagnostic tests are not available in most laboratories in China and only *L. pneumophila* serogroups 1 to 6 have been studied.

Sera from 319 healthy individuals living in Taiyuan, China were tested with 15 legionella antigens, which included *L. pneumophila*, serogroups 1 to 6, *Tatlockia* (*Legionella*) *micdadei, Fluoribacter* (*Legionella*) *bozemanae, Fluoribacter dumoffii, Fluoribacter gormanii, "Legionella" anisa, Legionella oakridgensis, "Legionella" longbeachae*, (serogroups 1 and 2), and *Legionella jordanis*. Two clusters were noted and seroreactivity to the legionellae appeared to be as prevalent as in other countries studied.

Materials and Methods

Serum samples and epidemiologic data: In January 1986, serum specimens were collected from 319 healthy volunteers living within the urban area of Taiyuan, China. Volunteers were solicited from four sites: the Shanxi Mining College, the Shanxi Daily newspaper, the Bureau of Mining dormitory, and an electric power plant. Their ages ranged from 18 to 66 years, (mean 37 years); 49% were male, and 33% were smokers. In October 1986, a second serum sample was collected from 39 who were available; their average age was 33 years, 52% were males and 32% smoked. Date forms completed for each individual recorded their age, sex, profession, work site, smoking history, residence, and history of recent illness. All specimens were assayed for IgG antibodies against the various antigens; 86 sera were also tested for IgM antibodies. Statistical significance was estimated by the Chi-square test. The association between prevalence and sex, occupation or smoking was tested after controlling for confounding factors using Mantel-Haenzel Chi-square statistics.

Enzyme-linked immunosorbent assay (ELISA): Bacterial antigens were prepared from each of the strains listed in Table 1. Agar-grown bacteria suspended in sterile phosphate-buffered saline pH 7.2 (PBS) were heat killed, washed, and resuspended in sterile PBS (0.1 g wet weight of bacteria per 1.5 ml). The bacterial suspensions were sonicated with five 30-sec bursts of a Branson sonifier at full power at 4°C in a biological safety cabinet. The supernatants, after centrifugation at 25,000 g for 20 min, were diluted to a protein concentration of 0.2 mg/ml, and then stored as aliquots at - 20°C for subsequent use as stock anti-

Received: 28 October 1987/Accepted: 24 April 1988

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Table 1: Bacterial strains used.

Species	Strain	Serogroup	Source
Legionella pneumophila	Philadelphia 1	1	CDC
	Togus 1	2	CDC
	Bloomington 2	3	CDC
	Los Angeles 1	4	CDC
	Dallas 1 E	5	CDC
	Chicago 2	6	CDC
Tatlockia micdadei	TATLOCK		CDC
Fluoribacter bozemanae	WIGA	1	*A.W. Pasculle*, Presbyterian Univ. Hosp., Pittsburgh, PA*
Fluoribacter dumoffi	NY-23		*A.W. Pasculle*, Presbyterian Univ. Hosp., Pittsburgh, PA*
Fluoribacter gormanii	LS-13		*A.W. Pasculle*, Presbyterian Univ. Hosp., Pittsburgh, PA*
Legionella (Fluoribacter) anisa	E-327F		*P. Edelstein*, Wadworth VAMC – Los Angeles, CA
Legionella longbeachae	Long Beach 4	1	CDC
	Tucker 1	2	CDC
Legionella oakridgensis	OR-10		CDC
Legionella jordanis	ABB9		CDC

* From CDC derived stocks

gen. Sonicates of *Pseudomonas aeruginosa* (ATCC 27853) were similary prepared.

Polystyrene microtitration plates (U-bottom, Dynatech Laboratories) containing 100 µl per well of stock antigen diluted 80-fold in PBS were incubated for at least 3 h at 37°C. The plates were then washed in a Titertek Multiwash (Flow Laboratories) with PBS containing 0.05% Tween-20 (PBS-T). The sera were diluted 1:10 with concentrated *P. aeruginosa* sonicate, and incubated at 37°C for 30 min; subsequent dilutions were made in PBS-T containing 0.5% bovine serum albumin (PBS-T-BSA). After incubating the diluted sera for 1 h at 37°C in the antigen coated wells, the plates were again washed with PBS-T and 100 µl of a 1:800 dilution (in PBS-T-BSA) of horseradish peroxidase-conjugated rabbit-anti-human γ- or µ-chain (Cappel Laboratories) was added to each well. After incubation for 1 h (37°C), the plates were washed, and 100 µl of a 0.01% o-phenylenediamine and 0.003% hydrogen peroxide solution was added. After incubation at 37°C for 40 min, the reaction was stopped with 50 µl 8N H_2SO_4. After 15 min, serum titer endpoints (A = 0.25) were read in a Titertek Multiscan (Flow Laboratories) using filter #4(492 nm).

The ELISA used in these studies differs from that which we previously used (4) as follows: a sonicated antigen was used to obtain better, more reproducible antigen yields, the concentration of BSA in the reagents was increased from 0.05% to 0.5% to decrease non-specific protein binding, and the *P. aeruginosa* sonicate diluent step was added to decrease cross-reactivity with non-specific antigens (5). By comparing the methods with known positive and negative sera (4,5), IgM titers of ≥ 1:320 and IgG titers of ≥ 1:640 were found to predict recent infection with *L. pneumophila* serogroups 1 and 6 and *T. micdadei*. Although similar data were not available for the other antigens, the same end points were used. In order to ascertain the reactivity of these "unstandardized" antigens, control "positive" sera were found through screening of patients with recent pneumonia consistent with legionellosis; most were cross-reactive with several legionella antigens.

Results

Of the 15 antigens tested, significant antibody levels were detected to 11, most commonly Philadelphia 1 (7.2%); ABB9 (3.1%), Togus 1 (2.8%), E-327 F (2.8%) and Los Angeles 1 (2.5%). Eight of 86 specimens tested contained IgM antibody in titers of 1:320 or more against NY-23, LS-13, WIGA or TATLOCK; four of these sera were also positive for IgG antibody.

Reactivity with any antigen was, in general, correlated with reactivity to others ($X^2 \geq 10.5$, df=1, $p < 0.005$). Cross-reactivity was even more strongly correlated when comparing response to antigens within genera or species (i.e. among *L. pneumophila* serogroups, *L. longbeachae* serogroups, or *Fluoribacter* species; $X^2 \geq 74.7$) The most strongly correlated pairs are indicated in Table 2 ($X^2 \geq 131.2$). The 83 reactive individuals reacted to an average of 3.6 antigen groups, or 4.9 individual antigens. When reactivity to these "natural" groups was studied (see Table 2), the most common reaction was still to Philadelphia 1, followed by E-327F/NY-23 (5.0%), Los Angeles 1/Dallas 1E (3.8%), Togus 1/Bloomington 2 (3.5%), and ABB9 (3.1%). Four sera were so broadly cross-reacting that they were considered to be "untypeable".

The prevalence of legionella antibodies was highest in those 30 to 39 years of age (31.3%) and lowest in those over 49 (23.8%). There was no significant difference between the prevalence in males (26.1%) and females (25.9%), even after controlling for smoking and occupation. The rate of reactivity to *L. pneumophila* serogroup 1 was highest in workers and staff at the power plant and the dormitory; E327/NY-23 reactivity was highest among students and staff at the college (Table 2). While the over-

Table 2: Seroprevalence by worksite and antigen.

Site	No. of individuals tested	Reactive with antigen(s)								
		Philadelphia	Togus & Bloomington	Los Angeles & Dallas	E327F & NY23	ABB9	WIGA & LS13	Long Beach & Tucker	Other	Total reactive (any antigen)
Shanxi Mining College	105	6(6)[a]	4(4)	0	10(10)	3(3)	3(3)	8(8)	2(2)	25(24)
Shanxi Daily newspaper	58	4(7)	5(9)	4(7)	4(7)	3(5)	3(5)	3(5)	4(7)	15(26)
Bureau of Mining dormitory	14	3(21)	1(7)	0	0	1(7)	1(7)	1(7)	1(7)	4(29)
Electric power plant	142	16(11)	7(5)	7(5)	6(4)	7(5)	5(3)	8(6)	13(9)	39(28)
Total (all sites)	319	29(9)[b]	17(5)	11(3)	20(6)[b]	14(4)	12(4)	20(6)	20(6)	83(26)
GMT[c] (reactive sera)	83	1067	758	735	897	705	616	732	578	
GMT (all sera)	319	414	348	343	378	336	224	307	--	

a Numbers in parentheses indicate percentage of individuals tested at that site who were reactive;
b The percentage reacting to Philadelphia 1 antigen is 7% when omitting the power plant employees, and the percentage reacting to E-327F/NY-23 is 5% when Shanxii Mining College is omitted;
c GMT = geometric mean titer.

all prevalence of reactivity to one or more antigens was 33.0% and 23.1% in smoking and non-smoking groups, respectively, this was not statistically significant. However, after controlling for sex and occupation, statistical significance was reached ($X^2 = 4.65$, df=1, $p < 0.05$).

Of the 39 individuals from whom a second serum sample was obtained ten months after the first specimen, titers in 37 were unchanged. Two individuals while unreactive ($\leq$ 1:320) with any antigen initially, became reactive at a dilution of 1:1280 against TATLOCK antigen in the second specimen. These seroconversions were, however, not associated with any recognized illness.

Discussion

The prevalence of reactivity to *L. pneumophila* serogroup antigens in China varied from 0.58% in Beijing and 1.5% in Wu Xi (3) to 28.72% in Tianjing (6). Our finding of 7.2% reactivity with *L. pneumophila* serogroup 1 antigen is similar to the rate observed in Nanjing (8.3%) and Hunan (9.9%) (3). In the United States, the prevalence of antibodies in individuals not associated with outbreaks varied from 1.7% to the Philadelphia-1 strain of *L. pneumophila* alone to 12% with one or more of 29 antigens (using the indirect immunofluorescence test, IFA, with end points $\geq$ 1:64 and $\geq$ 1:256, in the respective studies (7, 8). On the other hand, 5.7 to 19% of controls living or working at or near known outbreak sites were found to have significant antibody titers ($\geq$ 1:128) against *L. pneumophila* antigens (5, 9–13).

The prevalence of anti-legionella antibody in a community does not seem to predict either the potential for, or existence of, an outbreak. In Nottingham, England, where epidemic disease had occurred, the sera of only 0.1% of residents had IFA titers $\geq$ 1:128 (12), while in New Zealand, where only sporadic cases have been reported, 2.8% of individuals had similar IgG titers and 4.0% had IgM titers $\geq$ 1:128 (14). The specificity of anti-legionella antibody tests has been questioned. For example, although 36% of Israelis studied had antibody levels $\geq$ 1:1280 against legionella antigens, these antibodies could be removed in almost half (particularly those who reacted with *F. bozemanae* antigens) by absorption of their serum with *Rickettsia typhi* antigens (15). Cross-reactivity has also been commonly observed. In one study, the sera of only 45% of individuals reacted primarily or entirely with a single antigen (8).

With regard to the serogroups or species responsible for infection in the United States, approximately 50% of "positive" sera reacted primarily with antigens of *L. pneumophila*, serogroup 1, another 30% with serogroups 2 to 6 and 10 to 11% with *T. micdadei*, *F. bozemanae* or *F. dumoffii* (16). In Denmark, 5.6% (45/728) of sera from pneumonia patients reacted with *L. pneumophila* serogroup 1 antigens, 3.8% (9/238) with *T. micdadei* antigens, 1.7% (4/238) with *F. bozemanae* antigens, and 1.4% (10/728) and 0.87% (3/238) with *L. pneumophila* serogroup 4 and 6 antigens, respectively (17).

Using the ELISA, we previously found that 3.6% of 2664 patients on admission to the Pittsburgh VA Medical Center had titers $\geq$ 1:160 to *L. pneumophila* serogroup 1, as did 7% of 129 hospital employees at that hospital (4). In a survey of 214 asymptomatic employees of the Cantonal Hospital in Basel, Switzerland, approximately 5% had titers $\geq$ 1:640 to one or more of ten antigens (*L. pneumophila* serogroups 1 to 6, *T. micdadei, F. bozemanae, F. dumoffii*, and "*L.*" *anisa* [unpublished observations]). Therefore, exposure to legionella antigens appears to be as prevalent in the industrialized areas of China as it is in other countries which have been studied. In addition, the distribution of serogroups or species may also be similar. Cross-reactivity makes clear interpretation difficult, as it may indicate exposure to diverse antigens,

or reactivity to common antigens (18). The use of purified or semi-purified antigen may reduce this ambiguity.

In this study one person with IgG antibody to *L. pneumophila* serogroup 1 had an episode of pneumonia one month before the serum sample was obtained. The other IgG- or IgM-positive individuals, including the two who seroconverted to TATLOCK antigens, had no history of pneumonia in the year prior to the study. This suggests that most of the antibody reactivity probably resulted from subclinical infection. In addition, as diagnostic tests for legionellosis are not available in most laboratories in China, many patients with mild disease may have been missed.

The prevalence of antibody to *L. pneumophila* serogroup 1 antigen was twice as high among workers at the dormitory and the power plant (12.0%), compared to the students and employees of the college and newspaper (6.1%). Conversely, the prevalence of antibody to E327F/NY-23 antigens was higher among those at the college and newspaper (8.6%), as opposed to those at the other sites (3.9%). While these differences do not quite reach statistical "significance", these trends and the differences in reactivity to Los Angeles and Dallas antigens (statistically significant at $p < 0.05$ Fisher's Exact Test) deserve further study. In addition, the geometric mean titer (GMT) of reactive sera to Philadelphia 1 or E327F/NY-23 antigens was slightly, but significantly, higher than the GMT with other antigens, which supports the suggestion that the differences in seroprevalence are due to local exposure to these organisms. The power plant, newspaper and dormitory are approximately 2 to 3 km from one another and are east of the Fan River, while the mining college is to the west of the river. Epidemiologic links have not yet been established between these facilities. A retrospective case-control study, prospective surveillance, and environmental studies are being initiated to further define the rates and mechanism of transmission of legionellosis in this area.

Acknowledgement

This work was supported by the VA Medical Research Service.

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