Legionnaires' disease – Results of a multicentre Canadian study

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BACKGROUND: There has never been a cross-Canada surveillance project to determine the rate of *Legionella* species as a cause of community-acquired pneumonia requiring hospitalization and to determine whether there are any regional differences in the rates of Legionnaires' disease in Canada. Anecdotally, Legionnaires' disease is thought to be uncommon in Western Canada.

METHODS: From January, 1996 through to October 31, 1997, a prospective study of the etiology of community acquired pneumonia requiring admission to 15 tertiary care hospitals in eight Canadian provinces was conducted. A urine sample from each patient was tested for *Legionella pneumophila* serogroup 1 antigen using a commercially available ELISA assay. A culture of sputum or other respiratory specimens for Legionellaceae was carried out at the discretion of the attending physician. Two hundred thirty-four patients had acute and 6-week convalescent serum samples tested for antibodies to *L pneumophila* serogroups 1 through 6 using an ELISA method.

RESULTS: 28 of the 850 patients (3.2%) had Legionnaires' disease; 18 of 823 (2.1%) were positive for *L pneumophila* serogroup 1 by urinary antigen testing. The rate of Legionnaires' disease, based on urinary antigen, at the Halifax site was higher than that at the other sites (seven of 163 patients versus 11 of 660 [P=0.04]). Of the 28 cases of Legionnaires' disease identified using all methods, 11 of 277 patients (3.9%) were enrolled from Western provinces versus 17 of 573 patients (2.9%) from Eastern provinces (P=nonsignificant).

CONCLUSIONS: Legionnaires' disease is just as common in Western as in Eastern Canada. *L pneumophila* serogroup 1 may be more common in Halifax than at the other sites studied.

Key words: Acquired pneumonia; Canada; Legionella; Legionella urine antigen; Legionnaires' disease

La maladie des légionnaires: Les résultats d'une étude canadienne multicentre

HISTORIQUE : Il n'y a jamais eu de projet de surveillance intercanadien pour déterminer le taux d'espèces de *Legionella* responsables de pneumonies non nosocomiales exigeant une hospitalisation et pour établir s'il existe des différences régionales dans le taux de maladie des légionnaires au Canada. Selon des rapports isolés, la maladie des légionnaires serait rare dans l'Ouest canadien.

MÉTHODOLOGIE : De janvier 1996 au 31 octobre 1997, a été menée une étude prospective de l'étiologie de la pneumonie non nosocomiale exigeant une hospitalisation dans l'un des 15 hôpitaux de soins tertiaires de huit provinces canadiennes. Un échantillon d'urine provenant de chaque patient a été examiné afin de détecter l'antigène de sérotype 1 de *Legionella pneumophila* au moyen d'un dosage ELISA commercial. Un prélèvement des expectorations ou d'autres échantillons respiratoires de Legionellaceae a été exécuté, selon le bon vouloir du médecin traitant. Les échantillons sériques de 234 patients ont été vérifiés pendant la phase aiguë et à la sixième semaine de la convalescence afin de détecter la présence des sérotypes 1 à 6 de *L pneumophila* au moyen de la méthode ELISA.

RÉSULTATS : Vingt-huit des 850 patients (3,2 %) souffraient de la maladie des légionnaires. Dix-huit patients sur 823 (2,1 %) étaient positifs au sérotype 1 de *L pneumophila* selon l'épreuve d'antigène urinaire. Le taux de maladie des légionnaires à l'établissement de Halifax, d'après l'antigène urinaire, était supérieur à celui des autres établissements (sept des 163 patients par rapport à 11 sur 660 [P=0,04]). Sur les 28 cas de maladie des légionnaires repérés au moyen de toutes les méthodes, 11 des 277 patients (3,9 %) ont été enrôlés dans les provinces de l'Ouest, par rapport à 17 des 573 patients (2,9 %) dans les provinces de l'Est (P=non significatif).

CONCLUSIONS : La maladie des légionnaires est tout aussi courante dans l'Ouest canadien que dans l'est du Canada. Le sérotype 1 de *L pneumophila* pourrait être plus courant à Halifax que dans les autres établissements étudiés.

Legionnaires' disease is an acute infectious disease of which the predominant manifestation is pneumonia (1). The most common cause is *Legionella pneumophila* serogroup 1, however, just under half of the over 40 recognized species in the Legionellaceae family can cause Legionnaires' disease (2). A soluble antigen is present in the urine of patients with Legionnaires' disease due to serogroup 1 (3-5). A

commercially available enzyme immunoassay is available that detects this antigen with a specificity of 100% and a sensitivity of 94.6% (3). We used this test to determine the frequency with which *L pneumophila* serogroup 1 was a cause of community-acquired pneumonia (CAP) in Canada, and to determine whether there was any geographic clustering of the cases of Legionnaires' disease. We tested a subset of the study

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population for *Legionella* species infection using a serological assay. Our objectives were to determine the rate of seroconversion among patients with a positive urinary antigen assay and to determine whether infection with serogroups 2 to 6 occurred in Canada.

MATERIALS AND METHODS

Patient populations and study design

This was a prospective observational study of CAP requiring initial hospitalization that began January 11, 1996 and ended October 31, 1997. It was conducted at 15 teaching hospitals in eight Canadian provinces. The study was approved by local ethics committees. Written, informed consent was obtained for each participant in accordance with national guidelines and the Declaration of Helsinki (Hong Kong amendment, 1989). All patients who were admitted to hospital with a diagnosis of pneumonia were screened by a study nurse. Patients who met the following eligibility criteria were approached for enrollment in the study: aged 16 years or older; a new pulmonary infiltrate on chest radiograph not attributable to an etiology other than pneumonia; two or more of the following symptoms or signs: chest pain, cough, rales, rhonchi and/or signs of consolidation, an oral temperature of less than 36.5°C or more than 38.5°C; and had not been hospitalized for at least 14 days. Sputum production was not a requirement for study entry, however, if it was ordered by the attending physician all attempts were made to collect a sample for Gram stain and culture. A study nurse completed the data collection forms and made daily visits (for a maximum of seven days follow-up while in hospital) to each patient to record progress in terms of evolution of the pneumonia and any complications that may have occurred during the hospital stay. All data were verified against the original chart by a study monitor during a site visit. Eight hundred fifty patients entered the study and all supplied informed consent.

Diagnostic testing

Blood and sputum specimens were collected at the discretion of the attending physician and processed for culture in accordance with the methodology in use at each centre's laboratory.

Acute phase serum samples were available on 837 patients and convalescent samples were available on 544 patients. All serum samples were tested for antibodies to *Mycoplasma pneumoniae*, Influenza viruses A and B, parainfluenza viruses 1, 2, and 3, adenovirus, and respiratory syncytial virus using a standard complement fixation technique in microtitre plates. The adenovirus and respiratory syncytial virus antigens were purchased from Flow Laboratories (USA); the influenza A and B, parainfluenza 1, 2, and 3, and *M. pneumoniae* antigens were purchased from MA Bioproducts (USA).

Serum samples from 539 patients were tested for Immunglobulin (Ig) G and IgM antibodies against *Chlamydia pneumoniae* AR-39 strain; *Chlamydia psittaci* – avian strain 6BC, feline pneumonitis strain FP, turkey strain TT3, pigeon strain CP3; *Chlamydia pecorum* – ovine polyarthrititis strain; and *Chlamydia trachomatis* – pooled antigens of serovars BED, CJHI, and FGK using the microimmunofluorescence assay (6). In addition, the 539 serum samples were also tested (272 had acute and convalescent serum samples tested and 267 had single serum samples tested) for antibodies to *Streptococcus pneumoniae* pneumolysin, pneumolysin immune complexes, C polysaccharide, surface protein A, *Haemophilus influenzae* and *Moraxella*

(*Branhamella*) *catarrhalis* (7-9). Antibodies to *Coxiella burnetii* phase I and II antigens were determined using a microimmunofluorescence assay as previously described (10).

Legionella species urinary antigen

Patients had urine collected at baseline for detection of *L. pneumophila* serogroup 1 antigen. An enzyme immunoassay was used according to the manufacturers instructions to detect this antigen (11). Testing was done in the microbiology laboratories of participating institutions.

Legionella species serology

A subset of the 850 patients had acute and convalescent serum samples tested for antibodies to *L. pneumophila* serogroups 1 to 6 at VY's laboratory in Pittsburgh, Pennsylvania using an enzyme immunoassay (12). A fourfold rise in antibody titre to at least 1:256 or a stable titre of at least 1:512 was considered to be diagnostic of *Legionella* species infection.

Statistical analysis

The data were entered into SPSS 8.0 (SPSS Inc, USA). Differences between patient subpopulations were tested using the Pearson χ^2 test, in the case of categorical variables, and the Student's *t*-test or one-way ANOVA, for continuous variables.

RESULTS

Twenty-eight of the 850 patients (3.2%) had a diagnosis of Legionnaires' disease made. This diagnosis was made on the basis of a positive urinary antigen test in 18 patients; on the basis of a positive culture in four patients (*L. pneumophila* serogroup 1: one patient; *L. pneumophila* serogroup 2: two patients; and *Legionella micdadei*: one patient). The remainder were diagnosed serologically (*L. pneumophila* serogroup 1: one patient; serogroups 2 to 6: five patients).

Two patients with serogroup 1 infection had a negative urinary antigen assay – one of these patients had the infection diagnosed serologically and the other had *L. pneumophila* cultured from respiratory secretions. The sensitivity of the urinary antigen test for the detection of a serogroup 1 infection was 90%.

Table 1 shows the site-specific positivity rates for *L. pneumophila* urinary antigen, which ranged from 0% to 7.6%. The latter occurred at the Quebec City site, where there was an outbreak of Legionnaires' disease associated with exposure to a contaminated cooling tower. The urinary antigen positivity rates were higher at the Quebec City site ($P < 0.04$) and at the Halifax site ($P < 0.04$) when compared with all the other sites. The St John's case was a truck driver from Nova Scotia who likely acquired his infection in Nova Scotia rather than in Newfoundland. When all 20 cases of serogroup 1 infection were considered, there was no difference in the rates of infection in Eastern versus Western Canada – 14 of 573 (2.4%) versus six of 277 (2.2%) ($P = \text{non-significant}$). Overall, there were eight cases of nonserogroup 1 infection. Five of these occurred among the 277 patients (1.8%) from Western Canada versus three among the 573 (0.5%) from Eastern Canada ($P = 0.07$). However, two of the serogroup 2 infections among patients from Eastern Canada (a husband and wife who had just returned to Toronto from a trip to Spain) were likely acquired in Spain. If these two

TABLE 1
Results of *Legionella pneumophila* serogroup 1 urinary antigen testing by site

Site	Province	Number tested	Number positive	Per cent positive
St John's	NF	64	1	1.5
Halifax	NS	163	7	4.29
Quebec	QC	39	3	7.6
Sherbrooke	QC	20	0	0
Montreal	QC	68	0	0
Toronto (2 sites)	ON	32	0	0
Ottawa	ON	86	0	0
London	ON	74	2	2.7
Winnipeg	MB	126	2	1.5
Saskatoon	SK	3	0	0
Edmonton	AB	75	2	2.6
Calgary (site 1)	AB	33	1	3
Calgary (site 2)	AB	31	0	0
Vancouver	BC	9	0	0
Total		823	18	2.18

AB Alberta; BC British Columbia; MB Manitoba; NF Newfoundland; NS Nova Scotia; ON Ontario; QC Quebec; SK Saskatchewan

cases are eliminated, the rates are 1.8% for patients from Western Canada versus one of 571 (0.017%) ($P=0.01$). If only those who had acute and convalescent serology tested for antibodies to *L pneumophila* serogroups 2 to 6 are considered, the rates were one of 150 (0.6%) for patients in Eastern Canada versus four of 84 (4.7%) for those in Western Canada ($P=0.057$).

Forty per cent of the *L pneumophila* serogroup 1 cases occurred during the months of July to September ($P=0.01$ for trend) (Table 2).

The 20 patients with *L pneumophila* serogroup 1 infection were compared with 224 patients who had a negative *Legionella* species urinary antigen test and negative serology tests for *L pneumophila* serogroups 2 to 6 (Table 3). Patients with Legionnaires' disease were more likely to present to hospital earlier after the onset of symptoms, to complain of myalgias and headaches, and to have a higher temperature at the time of admission than were patients with pneumonia due to other causes. They were less likely to have chronic obstructive pulmonary disease. There was no difference in mortality rate or length of stay.

Table 4 shows the results of serological testing on 10 patients with a positive urinary antigen test. Only three of the 10 had a positive serological test.

Fifteen of the 18 patients with a positive urinary antigen test had additional serological testing as described in the methods section. Seven patients (46.6%) had a copathogen identified. These included three cases of *Streptococcus pneumoniae* and one each of adenovirus, influenza virus A, influenza virus B, and *Mycoplasma pneumoniae*. Six of the 18 patients had blood cultures performed before antibiotic therapy and all were negative.

DISCUSSION

A Medline search from 1976 through 2001 revealed 33 papers dealing with *Legionella* species infection in Canada. Seventeen of these papers dealt with nosocomial

TABLE 2
Seasonal occurrence of 20 cases of *Legionella pneumophila* serogroup 1 infection compared with 830 cases of pneumonia that had a negative *Legionella pneumophila* serogroup 1 (other cases) urinary antigen assay

Months	Serogroup 1 (%)	Other cases (%)
January to March	3 (15)	308 (37)*
April to June	6 (20)	219 (26)
July to September	8 (40)	127 (15)
October to September	3 (15)	176 (21)

* $P=0.01$ for trend

TABLE 3
Comparison of selected features of 20 patients with *Legionella pneumophila* serogroup (SG) 1 infection with 224 patients who did not have *Legionella* species infection

Feature	<i>L pneumophila</i> SG 1	No <i>Legionella</i>	P
Mean age (years)	62.8±14.3	63.1±17.3	0.93
Male	16 (80%)	133 (59.4%)	0.09
Mean time (days) between onset of symptoms and admission	4.7±4.3	7.7±13.3	0.02
Chronic obstructive pulmonary disease	1 (5%)	80 (35.7%)	0.005
Myalgias	12 (60%)	85 (38%)	0.001
Headache	9 (45%)	40 (17.9%)	0.007
Diarrhea	6 (30%)	42 (18.7%)	0.24
Mean oral temperature (°C) at admission	38.3±1.2	37.2±5.2	0.0001
Serum Na <136 mM/dL	12 (60%)	89 (39.7%)	0.09
Mean length of stay (days)	9.5±6.6	9.9±9.9	0.77
Died	2 (10%)	15 (6.7%)	0.63

Na Sodium

Legionnaires' disease. From this literature it is apparent that *L pneumophila* serogroup 1 and *Legionella longbeachae* serogroup 2 were present in the water supplies of Nova Scotia hospitals (13) and that *L pneumophila*, *Legionella micdadei* and *Legionella dumoffi* had been recovered from clinical and/or environmental specimens in the Quebec City area (14,15). *L pneumophila* serogroups 1, 3, 6 and 12, *Legionella saintelensis*, and *Legionella feelei* have been reported from Ontario (16-21); *L pneumophila* serogroup 1 from Manitoba (22); and *Legionella maccacchermii* from Saskatchewan (23). Nicolle et al (24) reported nine patients with community-acquired Legionellosis who were hospitalized at the Health Sciences Centre in Winnipeg between 1986 and 1991. Eight of these infections were due to *L pneumophila* serogroup 1 and one was due to *L pneumophila* serogroup 6. The current study is the first to try and determine the importance of *Legionella* species as a cause of CAP in Canada. We found that 2.1% of the patients were infected with *L pneumophila* serogroup 1. This infection was more common in Halifax and Quebec City than elsewhere; however, the Quebec City cases were related to an outbreak secondary to exposure to a contaminated water cooling tower. When all

TABLE 4
Results of serological testing (ELISA) of patients with a positive urinary antigen for *Legionella pneumophila* serogroup 1

City (patient number)	Acute IgM/IgG	Convalescent IgM/IgG	Time between acute and convalescent testing (weeks)
St Johns (31)	neg	neg	7
Winnipeg (16)	neg	neg	5
Winnipeg (100)	>512/>512	>512/>512	10
London (35)	neg	neg	7
Halifax (48)	neg	neg	5
Halifax (54)	neg	neg	12
Halifax (72)	neg	neg	4
Qeubec (15)	<64/<64	256/256	7
Quebec (16)	<64/<64	128/>512	8
Qeubec (18)	neg	neg	8

Ig Immunoglobulin; neg Negative

28 cases of Legionnaires' disease were considered, cases were just as common in Western Canada as in Eastern Canada. It appeared that cases of *L pneumophila* serogroups 2 to 6 were more common in Western Canada when two of the three cases in Eastern Canada were removed from the analysis (these two cases appeared to be acquired in Spain). Our study has several limitations regarding conclusions about *Legionella* species infections in Canada: cultures of respiratory secretions for *Legionella* species were not done routinely, and only 234 patients had serological testing for *L pneumophila* serogroups 1 to 6.

Early in the course of study of Legionnaires' disease a summer-fall predominance of cases was noted (25). We noted the same seasonality.

In one of the better studies of the *Legionella* species urinary antigen test, Plouffe et al (5) noted a specificity of 99% and an overall sensitivity of 56%. Our overall sensitivity of 64% is in keeping with Plouffe's findings. Seven of 10 patients with positive urinary antigen in our study had negative acute and convalescent serology. Does this mean that the urinary antigen tests were false positives or that the serological tests were falsely negative? Failure of seroconversion of culture-positive cases of Legionnaires' disease is well known even when serum samples are obtained up to 10 or 12 weeks convalescent (26). Indeed the sensitivity of serology ranges from 60% to 75% (26), thus it is possible that some of our results represent false-positive urinary antigen tests. We also documented two false-negative urinary antigen tests.

Sopena et al (27) compared 48 patients with Legionnaires' disease with 125 patients with pneumonia due to other causes. By multivariate analysis the patients with Legionnaires' disease were more likely to have no underlying disease, to complain of diarrhea, and to have an elevated creatine phosphokinase. Mulazimoglu and Yu (28) reviewed 14 studies of the etiology of CAP to try to determine the clinical features that distinguish Legionnaires' disease from other causes of pneumonia. They only included studies that used two methods to diagnose *Legionella* species infection and excluded studies that relied only on serology. They found that headache, diarrhea, neurological symptoms (especially confusion), fever of more than 39°C, hyponatremia, elevated

creatinine phosphokinase, and abnormal liver function tests were the most consistent features in distinguishing Legionnaires' disease from other etiologies of CAP. We also found that headache was more common among patients with Legionnaires' disease than among patients with other causes of pneumonia. We noted that myalgias, a higher mean oral temperature, and the lack of chronic obstructive lung disease were more common among the patients with Legionnaires' disease. We also noted that patients with Legionnaires' disease presented to hospital sooner after onset of symptoms than did patients with other causes of pneumonia.

In our study the mortality rate for *Legionella* species serogroup 1 infection was 10% and for nonserogroup 1 infection it was 25%. Heath et al (29) studied 39 patients with Legionnaires' disease (36 were community-acquired) and found that the mortality rate was 26%. They noted that survivors had therapy with erythromycin started a median of six days from onset of symptoms compared with 11 days for those who died. We found that the median time from onset of symptoms until presentation to a hospital was 4.7 days for those with *L pneumophila* serogroup 1 infections. Most of our patients with *Legionella* species infection were treated with erythromycin, although four received azithromycin dihydrate. Edelstein (30) found that azithromycin dihydrate is more active against intracellular *L pneumophila* than erythromycin. He recommends treatment of Legionnaires' disease with a fluoroquinolone, which, in contrast to erythromycin, can kill intracellular *Legionella* species, or azithromycin dihydrate.

There are a couple of limitations to our study. Because of the requirements for informed consent, it is possible that enrollment was biased toward patients who were not seriously ill and, hence, less likely to have Legionnaires' disease. However, the overall mortality rate was 9.5%, which is similar to that in population-based studies (31,32). Another limitation is that the only test for Legionnaires' infection given to the entire population was the urinary antigen test.

CONCLUSIONS

Legionella species infections constitute a small percentage of cases of CAP requiring admission to hospital in Canada. These cases occur throughout Canada, and a comprehensive diagnostic approach is necessary for their diagnosis. It is not practical to carry out comprehensive testing for *Legionella* species infection on all patients who are admitted to hospital so this is best reserved for those in whom there is a high clinical suspicion of Legionnaires' disease, and those with rapidly progressive pneumonia. *L pneumophila* serogroup 1 infections may be more common in Halifax than in the rest of Canada. The higher rate of this infection in Quebec City was related to an outbreak. Infection with *L pneumophila* serogroups 2 to 6 is just as common as infection with serogroup 1.

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