Host-Related Risk Factors and Clinical Features of Community-Acquired Legionnaires Disease Due to the Paris and Lorraine Endemic Strains, 1998–2007, France

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Background. In France, Legionnaires disease is mainly caused by Legionella pneumophila. Here, we investigated possible host factors associated with susceptibility to community-acquired Legionnaires disease caused by the endemic Paris and Lorraine strains.

Methods. We conducted a double-nested exploratory case-control study with use of data from the French national surveillance network of incident Legionnaires disease cases notified from 1998 through 2007. Patients with community-acquired Legionnaires disease and an L. pneumophila serogroup 1 isolate were eligible. Case patients were patients infected by the Paris or Lorraine strain, and control patients were those infected by sporadic strains. Epidemiological and clinical factors associated with infection with the Paris and Lorraine strains were assessed by calculating adjusted odds ratios (aOR) in multivariate logistic regression models.

Results. We studied 1090 patients infected by sporadic strains (n = 920), the Paris strain (n = 80), or the Lorraine strain (n = 90). Infection with the Paris strain was significantly associated with female sex (aOR, 1.98; 95% confidence interval [CI], 1.19–3.28), steroid therapy (aOR, 3.16; 95% CI, 1.76–5.68), and a history of cancer or hematologic malignancies (aOR, 2.08; 95% CI, 1.15–3.76). In addition, the mortality rate was higher among patients infected with the Paris strain than in the control group (38% vs. 25.5%). The Lorraine strain was associated with smoking (aOR, 1.82; 95% CI, 1.14–2.91) and reduced mortality (9.9%).

Conclusion. Several host characteristics were associated with the risk of infection by endemic strains of L. pneumophila serogroup 1. These findings may help to guide preventive measures. Factors predisposing patients to infection by specific strains need to be explored further.

Legionnaires disease is a form of pneumonia secondary to inhalation of water aerosols containing legionellae. Legionella pneumophila is the species responsible for ~90% of cases, and serogroup 1 alone accounts for ~85% of cases [1, 2]. A diagnosis of Legionnaires disease can be made by serology, direct immunofluorescence, polymerase chain reaction, urinary antigen detection, or culture of clinical specimens; ~20% of confirmed Legionnaires disease cases in France are detected by culture [3]. In France, L. pneumophila serogroup 1 clinical isolates are genotyped to determine their sporadic, epidemic, or endemic nature [4]. Most cases are sporadic (i.e., they occur in epidemiologically unrelated patients) and are due to novel strains. A strain is considered to be endemic when several isolates have identical pulsed-field gel electrophoresis (PFGE) patterns and are responsible for several epidemiologically unrelated cases of Legionnaires disease across a country.
The first endemic strain of *Legionella* to be identified is the Paris strain, which was responsible for 8.2% of culture-proven cases of legionellosis from 1995 through 2006 [5, 6]. This strain is highly endemic in France and has also been linked to Legionnaires disease in Switzerland, Italy, Spain, Sweden, Senegal, Japan, and the United States [4, 5, 7]. Another endemic strain, Lorraine, emerged in the east of France in 2002 and accounted for 10.5% and 9% of French clinical isolates in 2005 and 2006, respectively [6].

Legionnaires disease remains a severe disease with a high case fatality rate [8]. Risk factors associated with the occurrence of Legionnaires disease include older age, male sex, smoking, a history of cancer or hematologic malignancies, steroid therapy, other immunosuppressive treatments, and diabetes mellitus [9–11]. Host genetic susceptibility to infection by *L. pneumophila* has also been identified. [12, 13]. However, no specific association between host factors and particular strains, as observed with *Pseudomonas aeruginosa* and *Escherichia coli*, has yet been demonstrated [14].

In this study, we crossed patients’ epidemiological data with *L. pneumophila* serogroup 1 genotyping data with use of the French national Legionnaires disease surveillance and national reference centre for Legionella (NRCL; Lyon, France) databases, respectively. The aim of this exploratory study was to determine whether, relative to sporadic strains, endemic strains of *L. pneumophila* (Paris and Lorraine) are associated with specific host characteristics.

**METHODS**

**Data sources.** In France, all cases of Legionnaires disease must be notified to the national health authority (Institut de Veille Sanitaire), which collects epidemiological information, including various risk factors for Legionnaires disease, through mandatory notification. In parallel, all corresponding *Legionella* isolates are sent to the NRCL, where they are characterized by PFGE. Strains are categorized as sporadic, epidemic, or endemic [4]. Adherence to the mandatory notification has not been evaluated recently, but it should have improved; for instance, the median time between disease onset and mandatory notification has been divided by 4 over 10 years (29 days in 1998 vs. 7 days in 2007). Cases with mandatory notification and reception of the corresponding isolate matched in 99.6% of cases in 2007.

**Design, population, and clinical data collection.** This observational study was based on prospective recruitment spanning a 10-year period from January 1998 through December 2007. Data were extracted from the French national Legionnaires disease surveillance database of notified cases [3]. Physicians and microbiologists are both required to provide notification (with use of a standard form) independently of all cases of Legionnaires disease to their local health authority, which in turn notifies the national surveillance institute (Institut de Veille Sanitaire). A code created based on date of birth, sex, first name, and initials of name is used to ensure patient anonymity. The form contains questions on current illness, the patient’s personal characteristics (sex and age), disease outcome, and factors known to be associated with Legionnaires disease (steroid therapy, other immunosuppressive therapies, history of cancer or hematologic malignancy, smoking, and diabetes mellitus) [15]. At the same time, *Legionella* isolates are systematically sent to NRCL for strain identification and molecular typing. All genotypes are compared with each other, each time 2 isolates have the same genotype, inquiries are performed to investigate the possible link between patients. Confirmed and probable cases of Legionnaires disease are defined as described by Campese and Decluit [16].

This analysis was restricted to patients with (1) community-acquired Legionnaires disease (excluding hospital-acquired infections but including infections associated with nursing homes and group homes), (2) radiologically confirmed pneumonia, and (3) *L. pneumophila* serogroup 1 isolated from a respiratory sample. For the purposes of this study, the population was stratified into 3 groups on the basis of infecting strains of *L. pneumophila* serogroup 1: (1) patients infected with sporadic strains, (2) patients infected with the Paris strain, and (3) patients infected with the Lorraine strain. All epidemiologically linked patients (clustered and outbreak cases) were excluded from the analyses, including those with infection due to Paris or Lorraine strains.

**L. pneumophila strains.** Each clinical isolate was plated on buffered charcoal-yeast extract agar supplemented with α-ketoglutaric acid (Oxoid). Serogrouping was performed by direct immunofluorescence with homemade antisera.

**Chromosomal PFGE analysis.** Genomic DNA was prepared as described elsewhere, with some modifications [5]. Briefly, *L. pneumophila* was treated overnight with proteinase K (50 μg/mL) at 55°C and was digested with 20 IU of SfiI restriction enzyme (Roche diagnostic) for 16 h at 50°C. Fragments of DNA were separated in 0.8% agarose gel in a contour-clamped homogeneous field apparatus (CHEF DRII system; Bio-Rad), with a constant voltage of 150 V. PFGE patterns were analyzed using GelComparII software (Applied Maths). The reference Paris strain was CIP 107629 (ST1), and the reference Lorraine strain was CIP 108729 (ST47) (Pasteur Institute strain collection). The PFGE patterns of the references strains were used for comparison.

**Statistical analysis.** Identification of duplicates between databases was based on the patient codes. Clinical and demographic characteristics were analyzed according to the 3 groups of *L. pneumophila* serogroup 1 strains. The χ² test and Fisher’s exact test were used, as appropriate, to compare endemic and sporadic strains [17].

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Factors associated with infection due to the Paris and Lorraine strains relative to that due to the sporadic strains were identified by calculating crude and adjusted odds ratio in univariate and multivariate logistic regression models. The variables considered for adjustment were sex, age, steroid therapy, immunosuppressive therapy, history of cancer or hematologic malignancy, smoking, diabetes mellitus, and year of notification. Final multivariable models were based on covariates identified by clinical judgment, in conjunction with automated variable selection methods (backward stepwise method according to selection criteria by the likelihood ratio method with \( P_{\text{in}} = .10 \) and \( P_{\text{out}} = .15 \)) [18]. The year of Legionnaires disease notification was systematically entered in the models. Final models were assessed for potential interactions and for adequate fit with the data with use of the Hosmer-Lemeshow goodness-of-fit statistic [19]. Analyses were conducted using SPSS software, version 12.0 (SPSS). P values were 2-sided, and the threshold of statistical significance was set at .05.

RESULTS

From 1998 through 2007, 9903 notified cases of Legionnaires disease met the case definition. A Legionella isolate was recovered in 1722 cases, and 1090 of these cases met the criteria mentioned above. The \( L. \ pneumophila \) serogroup 1 isolates comprised 920 sporadic strain isolates (84.4%; with unique PFGE patterns), 80 Paris strain isolates (7.3%; with PFGE patterns identical to that of the reference strain CIP 107629), and 90 Lorraine strain isolates (8.3%; with PFGE pattern identical to that of the reference strain CIP 108729) (figure 1). Two hundred fifty-nine isolates linked to outbreaks were excluded from the analysis. Among these 1090 cases, the annual number of all notified cases of Legionnaires disease varied over the study period, ranging from 32 (2.9%) notified cases in 1999 to 186 (17.1%) in 2005 (figure 2A). However, the proportion of Legionnaires disease cases with \( L. \ pneumophila \) isolation selected per year remained stable at ~10% of all notified cases (figure 2B). The median age of patients was 56 years (range, 19–100 years), and 856 patients (78.5%) were men. Outcome was unknown in 226 cases (20.7%). There were 218 deaths (20.0%), and 646 patients (59.3%) recovered. One hundred eighty-four of the patients who died were infected by sporadic strains (crude mortality rate, 25.5%), 27 by the Paris strain (crude mortality rate, 38.0%), and seven by the Lorraine strain (crude mortality rate, 9.9%). The crude mortality rate was significantly higher for those infected with the Paris strain than with sporadic strains (\( P < .001 \)), whereas it was lower with the Lorraine strain than with sporadic strains (\( P = .003 \)).

Female sex, advanced age, steroids or other immunosuppressive therapies, and a history of cancer or hematologic malignancy were more frequent among patients infected by the Paris strain, whereas smoking was more frequent among patients infected by sporadic strains (table 1). Smoking was more frequent among patients infected by the Lorraine strain than among patients infected by sporadic strains. After adjustment (table 2), infection by the Paris strain was independently associated with female sex (\( P = .008 \)), steroid therapy (\( P < .001 \)), a history of cancer or hematologic malig-

![Figure 1. Population chart.](https://academic.oup.com/cid/article-abstract/49/2/184/402261)
nancy \( (P = .015) \), and less smoking \( (P = .003) \). Smoking \( (P = .013) \) was the only host factor associated with infection by the Lorraine strain.

**DISCUSSION**

From 1998 through 2007, the clinical and demographic characteristics of French patients infected by the endemic *L. pneumophila* strains Paris and Lorraine were different from those of patients infected by sporadic strains. The Paris strain was positively associated with female sex, steroid therapy, and a history of cancer or hematologic malignancy and was negatively associated with smoking. The Lorraine strain was associated solely with smoking. The crude mortality rate associated with the Paris strain was higher than that associated with sporadic strains, whereas the Lorraine strain was less lethal than the sporadic strains. These results, based on logistic regression models, were confirmed with a polytomous logistic model (data not shown) [20].

Similar results have been reported for specific strains of other bacterial pathogens. O’Caroll et al. [14] reported that, among patients with cystic fibrosis, a clonal *Pseudomonas aeruginosa* strain (pulsotype 2) was associated with younger age and poorer lung function. Likewise, among 4 different phylogenetic groups (A, B1, D, and B2) of *Escherichia coli* isolated from patients with sepsis, Jauregui et al. [21] demonstrated that group B2 was independently linked to susceptibility to antibiotics, community-acquired infection, urinary tract origin, and normal immune status, whereas those isolates belonging to groups A, B1, and D were more frequently associated with resistance to antibiotics, hospital-acquired infection, non–urinary tract origin, and immunodeficiency.

A recent study compared epidemiological and clinical features, blood chemistry values, radiological findings, and outcome between sporadic and epidemic Legionnaires disease [22]. Patients with sporadic community-acquired Legionnaires disease were more frequently male and had a higher prevalence of chronic lung disease, HIV infection, and steroid therapy than did patients infected during outbreaks; moreover, sporadic cases were more severe and had a poorer outcome.

We found that patients infected by the Paris strain were more...
likely to have received steroids and to have a history of cancer or hematologic malignancy than were patients infected by sporadic strains. The higher susceptibility of such individuals to infection in general and the widespread presence of the Paris strain in the environment [4] suggest that the Paris strain may be well adapted to growth in the environment but is either less infective than sporadic strains or more immunogenic, thus triggering a more efficient host immune response. The particular tropism of the Paris strain for immunocompromised patients could explain the high prevalence of hospital-acquired Legionnaires disease caused by this strain (42.9% and 10% of hospital-acquired Legionnaires disease cases in France are caused by the Paris strain and sporadic strains, respectively [data not shown]).

Smoking was more frequently associated with the Lorraine strain than with sporadic strains. Smoking is a well-known risk factor for infection, particularly bacterial infections such as pneumococcal pneumonia, Legionnaires disease, and tuberculosis [23]. The underlying mechanisms are not fully understood. Host and/or bacterial factors may promote infection of smokers by the Lorraine strain. In contrast to the Lorraine strain, the Paris strain was less frequently associated with smoking than were sporadic strains. Gene polymorphisms or the absence of bacterial components involved in infection mechanisms influenced by smoking might explain the observed differences in the proportions of smokers between patients infected by the Paris and Lorraine strains. Alternatively, smoking might reflect another risk factor that was not explored in this study.

There was a difference in the sex ratios among patients infected with the Paris strain and those infected with sporadic strains (male-to-female ratio, 1.8 vs. 3.9). We have no explanation for this difference.

The crude mortality rate was higher among patients infected with the Paris strain than among those infected with sporadic
Table 2. Multiple logistic regression analysis of factors associated with infection by Paris and Lorraine strains of *Legionella pneumophila*.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Paris vs. sporadic (n = 1000)</th>
<th>Lorraine vs. sporadic (n = 1010)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude OR (95% CI)</td>
<td>Adjusted ORa (95% CI)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2.20 (1.36–3.56)</td>
<td>1.98 (1.19–3.28)</td>
</tr>
<tr>
<td>Malec</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;75</td>
<td>2.31 (1.31–3.05)</td>
<td>...</td>
</tr>
<tr>
<td>51–75</td>
<td>1.04 (0.54–2.00)</td>
<td>...</td>
</tr>
<tr>
<td>&lt;=50c</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td><strong>Steroid therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4.39 (2.52–7.65)</td>
<td>3.16 (1.76–5.68)</td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Other immunosuppressive therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.44 (1.10–5.42)</td>
<td>...</td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>...</td>
</tr>
<tr>
<td><strong>Cancer or hematologic malignancy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.11 (1.79–5.40)</td>
<td>2.08 (1.15–3.76)</td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.31 (0.19–0.52)</td>
<td>0.44 (0.26–0.76)</td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.07 (0.55–2.09)</td>
<td>...</td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; OR, odds ratio.

a Adjusted OR is from a logistic regression with Paris *Legionella* strain as dependent variable and with sex, steroid therapy, cancer or hematologic malignancy, smoking, and year of notification (variable not shown in the table) as covariates for adjustment. The Hosmer-Lemeshow goodness-of-fit statistic for the final model was 0.72 [19].

b Adjusted OR is from a logistic regression with Lorraine *Legionella* strain as the dependent variable and with smoking and year of notification (variable not shown in the table) as covariates for adjustment. The Hosmer-Lemeshow goodness-of-fit statistic for the final model was 0.99 [18].

c Reference group.

strains, but there were more missing data regarding death among patients infected with non-Paris strains than among patients infected with the Paris strain. Because of the advanced age and immunosuppression of the host population infected by the Paris strain, this is not surprising. Strain-specific virulence factors could also be involved. Lawrence et al. [5], who first described the Paris strain, observed no significant difference in mortality or in other criteria such as age, sex ratio, patients with at least 1 risk factor (malignancy, organ transplantation, immunosuppressive therapy, or AIDS), and mechanical ventilation by comparing patients infected by the Paris strain with those infected by other strains. Compare to our study, the population examined by Lawrence et al. [5] was quite different (smaller population size, hospital-acquired infections included, and restricted to Paris area); nevertheless, for all criteria common to both studies (sex ratio, hospital-acquired infections proportion, risk factors, and morality), a similar tendency could be observed (e.g., mortality 32% for the Paris strain and 24% for other strains in the Lawrence et al. study, compared with 33.8% and 20%, respectively, in our study). The absence of statistical significance in the Lawrence et al. study may be attributable to the small size of the population. In contrast, the Lorraine strain was less lethal than sporadic strains. This difference could also be attributable to host factors or bacterial characteristics. Most of the patients infected by the Lorraine strain were otherwise healthy, and this could have contributed to their better recovery. If these differences in lethality are confirmed, then early strain identification with molecular methods might help with prognostication and patient management.

Although patient recruitment for this study was prospective and covered the entire French population and a national surveillance network, a selection bias cannot be ruled out. Legionnaires disease notification is mandatory, but some cases may not have been notified [24] through the passive data collection system. Other cases may have been treated empirically without an etiological diagnosis.
We observed an increase in the number of notified Legionnaires disease cases during the study period, as reported elsewhere in Europe [25]. In France, this may reflect both better surveillance and better diagnosis. The clinical definition of Legionnaires disease was the same throughout the study, but microbiological methods became increasingly sensitive, especially with the advent of urinary antigen testing. This evolution was taken into account by adjusting for the year of notification in the multivariate regression models. The geographic distribution of strains and its potential link with specific risk factors were not included in the analyses; nevertheless, the distribution throughout the country of each of these 2 strains does not seem to influence their association with specific risk factors (data not shown). The possibility that the diagnostic approach was related to the strains and the risk factors is very low.

Approximately 80% of reported cases of Legionnaires disease were not included in the analysis because there were no culture isolates from the patients. This represents a possible selection bias if the 20% of culture-proven cases are not representative of the entire group; because of this possible bias, interpretations of this study’s results should not be extended to culture-negative Legionnaires disease cases. Recently, the European Working Group for Legionella Infection network had developed a typing method different from PFGE that is used in several European countries [26, 27]. This technique, based on amplification and sequencing of 7 genes, has been recently adapted to allow typing directly from clinical samples, without the need of isolates [28]. This method will be used in additional studies to investigate the possible difference between these 2 groups of patients.

Host genetic factors have also been linked to an increased risk of L. pneumophila infection. A single nucleotide polymorphism in the trl5 gene is associated with greater susceptibility, whereas 2 single nucleotide polymorphisms in the trl4 gene are associated with reduced susceptibility [12, 13]. We could not determine the status of these polymorphisms in our patients.

The most important finding of this study is that different host factors were associated with infection by the 2 strains of L. pneumophila that are endemic in France. Immune deficiency and smoking, respectively, appear to be the main risks factor for infection by the Paris and Lorraine strains. These findings are also relevant to other parts of the world, because the Paris strain is found worldwide [7] and strains with the same sequence type as the Lorraine strain have been reported elsewhere in Europe [29].

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References