Progress in diagnostics and prevention of Legionnaires' disease
IJzerman, Eddy Peter Frans

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CHAPTER NINE

Distribution of *Legionella pneumophila* Genotypes in Patients and Environmental Sources

Ed P.F. Yzerman¹, Jacob P. Bruin¹, Jeroen W. den Boer², Linda P. Verhoef², Kim W. van der Zwaluw³

¹Regional Public Health Laboratory Kennemerland, Boerhaavelaan 26, 2035 RC Haarlem
²Municipal Health Service Kennemerland, Westergracht 72, 2014 XA Haarlem
³National Institute of Public Health and the Environment, POB 1, 3720 BA Bilthoven

**EPIDEMIOLOGY**

Legionnaires’ disease is a pneumonia that is responsible for 4-8% of the community-acquired pneumonias and an unknown percentage of hospital-acquired pneumonias in The Netherlands (2, 6). Despite this relatively low incidence, Legionnaires’ disease receives a lot of attention because it is considered a preventable event with a stable mortality rate between 5-15% over the years (3). Legionellae are part of the microbial community of aquatic ecosystems (natural as well as manmade), which explains why legionellosis occurs worldwide. In many countries, Legionnaires’ disease is a notifiable disease. Epidemiological study results from the United States show that 90% of all Legionnaires’ disease cases are caused by *Legionella pneumophila* and that 72% are caused by *L. pneumophila* serogroup 1 specifically (5).

**A NATIONWIDE STUDY IN THE NETHERLANDS**

To compare the Dutch epidemiology with the figures in the United States, we conducted a prospective study that started in August 2002. This nationwide study consisted of sampling potential sources Legionnaires’ disease patients had been exposed to during their incubation period (2 to 10 days). For this purpose, we were given access to national notification data. Both confirmed and probable cases were included in the study. A confirmed case of Legionnaires’ disease was defined as a patient suffering from symptoms compatible with pneumonia who showed radiological signs of infiltration and had laboratory evidence of *Legionella* spp. infection. Laboratory evidence included isolation of *Legionella* spp. from respiratory secretions or lung tissue, detection of *L. pneumophila* antigens in urine, seroconversion or a fourfold or higher rise in antibody tites to *L. pneumophila* in paired acute- and convalescent-phase sera. A probable case of LD was defined as a
patient suffering from symptoms compatible with pneumonia who showed radiological signs of infiltration and had laboratory findings suggestive of *Legionella* spp. infection. These findings included a high antibody titre to *L. pneumophila* in a single serum, direct fluorescent antibody staining of the organism and detection of *Legionella* species DNA by polymerase chain reaction in respiratory secretions or lung tissue. Both definitions conform to the criteria of the European Working Group for *Legionella* Infections (EWGLI) (1). Patients were excluded from the study if they were abroad during the incubation time. All 39 municipal health services of The Netherlands participated in the study by incorporating the study procedures into their standard notification protocol. A uniform questionnaire was used by all municipal health services to register the potential sources for each patient. The questionnaire facilitates a structured interview focused on individual exposure to potential sources of infection. Trained personnel from our laboratory subsequently sampled these sources systematically and cultured the water and swab samples according to standard procedures. In short, the water samples were concentrated by filtration and the filtered residues were resuspended in 1 ml sterile water. From this suspension, 100-microliter samples were cultured without dilution and after 10- and 100-fold dilution on buffered charcoal yeast extract agar, supplemented with α-ketoglutarate (BCYE-α) agar at 37°C, with increased humidity. In cases of bacterial overgrowth, cultures were repeated after pre-treatment by heating 30 minutes at 50°C. Swab samples were dispersed by immersion in 1 ml sterile water and cultured as described above. Isolates were identified biochemically; after identification, *Legionella* strains were serotyped using commercially available kits containing antisera against the fourteen *L. pneumophila* serogroups and *L. dumoffii, L. gormanii, L. micdadei, and L. bozemanii*. If they belonged to serogroup 1, the strains were genotyped by AFLP (amplified fragment length polymorphism) technique according to EWGLI (European Working Group for *Legionella* Infections) typing protocols (4). Upon report of a notified culture-proven patient, the medical microbiology laboratory involved was requested to send the patient isolate to our laboratory for sero- and genotyping. Patient and environmental strains were compared, and a potential source was considered to be a true source of infection if the patient isolate and an environmental isolate were indistinguishable by AFLP genotyping. Furthermore, for all isolated strains, we compared the distribution of genotypes causing disease to the distribution of environmental strains in order to investigate the possibility of targeting preventive measures.

**Distribution of Legionella Genotypes**

In this chapter we present the preliminary results of the distribution of *Legionella* genotypes cultured from patients and environmental sources. Between August 2002 and September 2005, sero- and genotyping of 130 patient isolates and 220 environmental isolates showed that 98% of the patient strains were from the *L.*
pneumophila genus. Of these, 87% belonged to L. pneumophila serogroup 1, 6% to serogroups 7-14, 3% to serogroup 3, 2% to serogroup 2 and 2% to serogroup 6 (Table 1). The most frequently seen EWGLI types were 004 Lyon (21%) and 010 London (10%) (Figure 1). Another 28% consisted of a mixture of AFLP types not yet designated by EWGLI. In contrast, a minority (45%) of environmental strains was of the L. pneumophila genus. Of this 45 percent, the distribution was 57%, 24%, 8%, 6%, 4%, and 1% for serogroups 1, 7-14, 5, 3, 2 and 6, respectively. The most frequently seen EWGLI types were 028 Rome (35%), 001 Lugano (13%), 013 London/030 Stockholm (13%) and 003 Glasgow (10%). The not-yet-designated AFLP patterns represented 17% of the environmental strains. Comparing the EWGLI types of the L. pneumophila serogroup 1 patient strains with the environmental strains, we noted that the distribution is completely different for both groups of strains. The genotypes 004 Lyon and 010 London, responsible for almost one-third of all Legionnaires’ disease patients in our study period in The Netherlands, were not found in the environmental samples collected from the potential sources provided by the patient in the structured interviews. On the other hand, the genotype 028 Rome was often present in the environmental cultures but only rarely cultured in patients.

Table 1. Distribution of L. pneumophila strains from patients and the environment, by serogroup (SG)

<table>
<thead>
<tr>
<th>SG</th>
<th>Patient strains number (%)</th>
<th>Environmental strains number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>112 (87)</td>
<td>56 (57)</td>
</tr>
<tr>
<td>2</td>
<td>2 (2)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>3</td>
<td>4 (3)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>8 (8)</td>
</tr>
<tr>
<td>6</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>7-14</td>
<td>8 (6)</td>
<td>24 (24)</td>
</tr>
</tbody>
</table>

ESTIMATION THE RISK OF HUMAN INFECTION

The preliminary results of our study indicate that systematic collection and sampling can provide insight into the distribution of the L. pneumophila genus and its serotypes and genotypes in humans and in the environment. Our data suggest that aquatic ecosystems (manmade or natural) colonised with Legionella strains represent a differentiated risk of causing Legionnaires’ disease depending on the presence of L. pneumophila versus L. non-pneumophila strains and then within the pneumophila group, depending on the genotype. Using the distribution as an a priori chance of occurrence, it may help to estimate the risk for human infection. This implies that genotyping the L. pneumophila strains isolated from
environmental sources can be used as a tool to fine-tune control measures. Based on our findings, actions in The Netherlands should be more aggressive for the EWGLI genotypes 004 Lyon and 010 London. However, until now, these genotypes have only rarely been isolated from environmental samples of potential sources. Legionellae are capable of infecting humans via aerosol inhalation or through drinking and subsequently aspirating water. For each patient in this study, an inventory of potential sources was drawn up based on this knowledge. The results indicate, however, that the inventories were incomplete. This raises the intriguing question of which sources were overlooked. Ongoing study may resolve this question in the future.

REFERENCES


