CHAPTER EIGHT

Outbreak detection and secondary prevention of Legionnaires' disease: A national approach

Jeroen W. Den Boer\textsuperscript{a}, Linda Verhoef\textsuperscript{a}, Max A. Bencini\textsuperscript{b}, Jacob P. Bruin\textsuperscript{b}, R. Jansen\textsuperscript{b}, Ed P.F. Yzerman\textsuperscript{b}

\textsuperscript{a}Municipal Health Service Kennemerland, P.O.Box 5514, 2000 GM Haarlem, The Netherlands
\textsuperscript{b}Regional Public Health Laboratory Kennemerland, Haarlem, The Netherlands

ABSTRACT

Background
To stop a possible outbreak of Legionnaires' disease (LD) at an early stage an outbreak detection programme was installed in The Netherlands.

Methods
The programme consisted of sampling and controlling of potential sources to which LD patients had been exposed during their incubation period. Potential sources were considered to be true sources of infection if two or more LD patients (cluster) had visited them, or if available patients' isolates and environmental Legionella spp. were indistinguishable by amplified fragment length polymorphism genotyping.

Results
Rapid sampling and genotyping as well as cluster recognition helped to target control measures. Despite these measures, two small outbreaks were only stopped after renewal of the water system. The combination of genotyping and cluster recognition lead to 29 of 190 (15%) patient-source associations.

Conclusion
Systematic sampling and cluster recognition can contribute to outbreak detection and lead to costeffective secondary prevention of Legionnaires' disease.

INTRODUCTION

Legionnaires' disease (LD) is an acute pneumonia of low incidence (9,22) which was first described after a large outbreak in Philadelphia in 1976 among visitors of a legionnaires' convention held in a hotel, and passers-by at the same hotel (13). The outbreak was shown to be caused by a newly discovered genus: Legionella spp. (20). These Gram-negative bacilli are ubiquitous in (manmade) aquatic environments and are capable of infecting humans by aerosol inhalation or by
drinking and subsequent aspiration of water. The omnipresence of *Legionella* spp. in water is in sharp contrast to the low incidence of LD. This contradiction has led to different theories on transmission, prevention and outbreak detection of LD. Outbreak detection became a topic of national interest in The Netherlands after an outbreak in 1999 involving 188 LD patients (12). An early warning system based on unexpected numbers of LD patients was introduced, leading to an alarm in the summer of 2002 when the incidence rate had doubled. Unfortunately, after thorough evaluation, no cluster or common source of infection could be identified (8). Shortly thereafter, a national outbreak detection programme was installed, aimed at a short response time between diagnosis and inspection and sampling of potential sources of infection. The programme was based on the observation that outbreaks are often preceded and followed by small clusters or solitary LD cases (5). Therefore, a source identification unit sampling potential sources was made available to all 39 regional public health services of the country. Officials of the Ministry of the Environment who supervised elimination of potential sources of infection assisted this unit. This action was expected to stop developing outbreaks at an early stage. Furthermore, by eliminating potential sources the outbreak detection programme would contribute to LD incidence reduction. These expectations were based on the observation that apparently sporadic cases of LD that appear over time are in fact clustered around the same source of infection (7). The longest time span covering such a cluster is 17 years (17). Source identification and subsequent elimination would thus prevent new LD cases to occur. To our knowledge, a national outbreak detection programme for Legionnaires' disease has not been tried before. This article describes the results of the first 2 years following installment in The Netherlands.

MATERIALS AND METHODS

*Inclusion of patients*

A confirmed case of LD was defined as a patient suffering from symptoms compatible with pneumonia, with radiological signs of infiltration, and with 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 four-fold or higher rise in antibody titres 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, with radiological signs of infiltration, and with 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. These definitions are
conform to the criteria of the European Working Group for *Legionella* Infections (EWGLI) (1).

Legionnaires’ disease (LD) has been a notifiable disease in The Netherlands since 1987. Treating physicians report LD patients within 24h to one of the 39 regional public health services in the country. Public health physicians subsequently report confirmed and probable LD patients within 24h to the Ministry of Health using an electronic disease notification system. This system was made accessible for this study. Patients were included in the study if the date of disease onset was between August 1, 2002 and July 31, 2004. Given the purpose of the study to identify Dutch sources of infection, all patients who had stayed abroad should be excluded. However, it is possible that some of these patients got infected with *Legionella* spp. after returning home. Arbitrarily, LD patients were also included if they had fallen ill 6-10 days after their return.

**Identification of potential sources**

The task of the regional public health services is to verify the diagnosis of reported LD patients and subsequently interview these patients (or their relatives) in order to identify potential sources of infection and to control a possible outbreak. Since 1990, all public health physicians use the same national protocol, which includes diagnostic criteria and a questionnaire. The questionnaire facilitates a structured interview focused at individual exposure to potential sources of infection. Inclusion of potential sources in the questionnaire was based on published results from epidemiological studies and outbreak reports. The potential sources of infection in The Netherlands for included LD patients that were identified using the questionnaire were reported to the research group and subsequently scheduled for sampling.

**Sampling of potential sources**

Trained laboratory personnel of the regional public health laboratory of Haarlem sampled potential sources. Both water and swab samples were collected. The water samples were concentrated by filtration and filtered residues were resuspended in 1ml sterile water. Of this suspension, 100 µl samples were cultured without dilution and after 10- and 100-fold dilution on buffered charcoal yeast extract supplemented with α-ketoglutarate (BCYE-α) agar at 37˚C, with increased humidity. In cases of bacterial overgrowth, cultures were repeated after pretreatment by heating 30 min at 50˚C. Swab samples were dispersed by immersion in 1 ml sterile water and cultured as described above. *Legionella* isolates were serotyped using commercially available kits: the *Legionella* Antisera "SEIKEN" Set (Seiken Denka Company Ltd., Tokyo, Japan, cat. nr. 311-701, which contains antisera against *L. pneumophila* serogroup 1, 2, 3, 4, 5, and 6 as well as antisera against *L. dumoffii*, *L. gormanii*, *L. micdadei* and *L. bozemanii*), and the Microscreen Kit (Microgen Bioproducts Ltd., Camberley, UK, cat. nr. M45, which contains antisera against *L. pneumophila* serogroup 1 and against *L. pneumophila*
serogroup 2-14). They were genotyped by amplified fragment length polymorphism, which is a whole-genome fingerprinting method that relies on the selective polymerase chain reaction amplification of restriction fragments (19). AFLP, which until recently (16) has been the method of first choice by EWGLI (15), is rapid and highly epidemiologically concordant ($E = 1.00$) but is not highly discriminatory ($D = 0.89$) (14). All environmental strains were serotyped and only if an available patient isolate was of the same serotype, genotyping was performed. From each environmental sample four colonies were picked for serotyping and subsequent genotyping. In case of an available patient isolate of a different serotype, an additional effort was made by picking up to 20 extra colonies for serotyping and subsequent genotyping.

If potential sources were sampled, priority was given to sources to which clusters could be attributed or to which large numbers of visitors were exposed. Potential sources were visited simultaneously by trained officials of the Ministry of the Environment who verified whether the water supply system was managed in accordance with legislative requirements (3). In short, owners of water supply systems are obliged to perform a risk assessment, take control measures and keep a logbook to administer preventive measures. If necessary, installations were put out of order and owners were given time to install control measures. These measures included removal of dead-end piping, regulation of water temperature and heat-flush procedures.

**Interpretation of sampling results**

A source identification result was considered:

1. negative if *Legionella* spp. were not cultured from the potential sources brought forward by the regional public health services,
2. inconclusive if *Legionella* spp. were cultured from one of the potential sources but neither a cluster could be identified nor matching fingerprints (see below) could be established,
3. positive if a cluster was identified or if matching fingerprints were established.

A cluster defined conforms to EWGLI criteria (2) as within a 2-year period two or more LD patients who were in their incubation period had visited the same potential source and this has come up in the structured interview. If two or more LD patients had residences less then 1 km apart and no common potential source was mentioned, they were also considered to fit the cluster definition. To indicate the lack of a specific common potential source, this type of cluster was named geographic cluster.

Matching fingerprints were defined as DNA-banding patterns induced by amplified fragment length polymorphism (19) of both patient-derived and source-derived *Legionella* spp. that could not be discriminated.
Statistical analysis

Statistical analysis was performed with version 12.0 of the SPSS statistical program (Chicago, IL, USA). Univariate analysis was used to identify factors associated with the presence of *Legionella* spp. in potential sources. The factors considered "possibly associated" were geographic region (north, east, south, west) and season (winter, spring, summer, autumn).

RESULTS

From 1 August 2002 through 31 July 2004, 446 LD patients were notified in The Netherlands. Of these, 188 patients who had stayed abroad and got ill before their return or within 5 days after their return were excluded. The remaining 258 patients fulfilled the criteria of inclusion, but 12 (5%) of them did not enter the study for various reasons. Apart from organizational constraints, the reasons cited most often were unwanted disclosure of private activities and fear for conflicts at the discovery of *Legionella* spp. in a potential source. The 246 patients who entered the study did not differ significantly in age, gender, fatal outcome, or geographic origin from the non-response patients. Of the patients in our study, 213 (87%) had suffered from confirmed LD, of whom 48 (23%) were diagnosed by culture of *Legionella* spp. from respiratory secretions or lung tissue. The remaining 33 (13%) suffered from probable LD. Table 1 shows their age and gender distribution.

The presence of *Legionella* spp. in environmental samples was significantly associated in univariate analysis with geography and season. An odds ratio of 2.2 (confidence interval (CI): 1.3-3.7) was calculated for the presence of *Legionella* spp. in samples originating from regions south and west versus north and south. Comparison of the presence of *Legionella* spp. in samples taken in winter and spring versus summer and autumn showed an odds ratio of 2.1 (CI: 1.2-3.5). The source identification result was negative for 129 LD patients (52%).

Table 1. Age and gender of 246 Legionnaires' disease patients included from 1 August 2002 through 31 July 2004 in The Netherlands

<table>
<thead>
<tr>
<th></th>
<th>Confirmed LD patients</th>
<th>Probable LD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Number (percentage)</td>
<td>145 (68%)</td>
<td>68 (32%)</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
<td>57.4 (11-84)</td>
<td>58-7 (5-92)</td>
</tr>
</tbody>
</table>

LD = Legionnaires' disease.
Table 2. Comparison of diagnostic results and positive environmental sampling results for 86 Legionnaires' disease patients for whom no matching DNA fingerprint was available

<table>
<thead>
<tr>
<th>Sampling results</th>
<th>L. pneumophila serogroup 1</th>
<th>L. pneumophila non-serogroup 1</th>
<th>Legionella non-pneumophila</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic results</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. pneumophila serogroup 1</td>
<td>5</td>
<td>5</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>L. pneumophila non-serogroup 1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Urinary antigen test</td>
<td>10</td>
<td>8</td>
<td>19</td>
<td>37</td>
</tr>
<tr>
<td>Four-fold rise or seroconversion</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Polymerase chain reaction</td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Single high titre</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Match = Genotypes of both patient-derived and source-derived Legionella spp. could not be discriminated by amplified fragment length polymorphism.

The source identification result was inconclusive for 86 (35%) of the LD patients (see Table 2). The inconclusive results include non-matching environmental Legionella spp. for 26 of 48 (54%) culture-positive LD patients. A substantial part (45%) of the environmental Legionella spp. was of the non-pneumophila genera. The source identification result was positive for 31 patients, giving an overall rate of 13% (31/246). The rate was 15% (29/190) for LD patients who had exclusively stayed in The Netherlands during their incubation period. In total, 17 clusters were discovered (sized 2-4 LD patients each) involving 29 patients. Twelve of these clusters would not have been identified timely without the outbreak detection programme. To 10 of the 17 clusters LD patients could be attributed who had attracted their disease prior to the study period. This information was reported spontaneously by LD patients or by public health officials and was verified using historical notification data of the Ministry of Health. A matching DNA fingerprint was found for six LD patients (2%). Given that Legionella spp. were cultured from environmental samples related to 32 of 48 (67%) culture-positive LD patients, a matching fingerprint was found in six out of 32 (19%) pairs. Excluding non-pneumophila environmental strains, a matching fingerprint was seen in six out of 20 (30%) pairs. Four of the six patients for whom a matching fingerprint was found had stayed in different hospitals during their incubation period, three of which had been associated with nosocomial LD in the past. One nosocomial LD patient had been using water from a water heater of which the temperature had been set at 35°C to prevent scalding incidents. The second nosocomial LD patient had regularly opened the window of a hospital room for fresh air. This room was situated within 10m of a cooling tower from which a matching L. pneumophila was sampled. The third nosocomial LD had stayed in an intensive care unit and the fourth in a private room at a bone marrow transplantation department. In the latter cases a matching L. pneumophila was isolated from a tap water faucet within 5m distance. Of the two community-acquired LD patients for whom a match was
established, the first had stayed in a mobile home during the entire incubation period. In contrast to his spouse this patient had not used the built-in shower. The second patient with community-acquired LD had stayed overnight in the house of his daughter for 4 days. In both cases *L. pneumophila* was cultured from a showerhead.

Control measures were taken at each potential source of infection as identified by public health officials using structured interviews. Despite these measures, 12 new patients occurred in relation to 10 potential sources. More rigorous measures were taken in order to stop transmission by these cluster-related sources. After these measures, no additional patients were reported in relation to all but two clusters. The first cluster consisted of LD patients who stayed at the same camping site. Eleven days after notification of the first patient *L. pneumophila* serogroup 1 was cultured from water samples originating from a shower unit on the camping site premises. Despite extensive control measures, two more patients (one with date of onset beyond the study inclusion period) reported after staying at this site. Only after renewal of the water supply system no additional patients were reported.

The second cluster that continued to expand was related to a water faucet in a hospital ward. Control measures were taken after the first patient. Despite these, two more LD patients (with dates of onset beyond the study inclusion period) could be attributed to the same faucet. Only after renewal of the existing water piping system, no more LD patients were reported.

DISCUSSION

Our study shows that active source identification can be successful on a national scale. Seventeen clusters were discovered of which 12 would not have been detected timely without a national identification system. The overall identification rate was 13% and 15% excluding international travellers. It seems that the benefits of including patients coming home from travel abroad do not justify the efforts. The identification rate of 15% is high compared to the pre-study period. In the pre-study period no procedures existed for the discovery of clusters involving more than one public health region. Small clusters went unnoticed and larger ones were discovered by chance. For example, a sauna-related LD outbreak of six patients over a period of 6 years involving five public health regions was only discovered when the sixth patient was presented at a regional meeting of public health officials in 1996 (10). The identification rate of 15% is lower than the 25% of EWGLI (21) and this is hardly surprising since the latter group of LD patients all comply to a well-documented environmental risk factor: spending one or more nights away from home (23,24).

The systematic implementation of control measures at each potential source associated with a LD patient must have had an impact on the elimination of sources of infection. However, despite these measures 10 clusters developed in the
course of our study. During the second visit of the source more rigorous preventive measures were advised. Still, on two occasions new LD patients could be attributed to the same source. The final and so far effective measure was complete replacement of the existing water supply system. This experience shows that source elimination not only depends on the speed of source detection. In the camping site outbreak *L. pneumophila* serogroup 1 was cultured from water samples 11 days after notification, whereas the water supply system was replaced 59 days after notification. These findings suggest that potential source elimination measures should be more rigorous and should be implemented within a shorter time period.

A total of six matching fingerprints were established. However, this does not necessarily imply that the thus identified source indeed caused disease. AFLP and other DNA-fingerprinting methods do not always distinguish between *Legionella* strains that have been cultured from sources hundreds of kilometres apart (4,18). Although not reported so far, in The Netherlands this phenomenon will be present as well. Apart from this restriction, the number of six matches that were established in our study does not seem very high. However, only 48 *Legionella* patient isolates were available meaning that for one in every eight LD patients a source of infection could be attributed with a relatively high degree of certainty (depending on the concomitant epidemiological evidence). The matching fingerprints teach us about the transmission patterns of LD and are therefore important in public health terms. Isolates were available from only 20% of the included LD patients in our study. Given this low rate, clinicians should be advised to make an effort to diagnose patients based on culture, even if the urinary antigen test or polymerase chain reaction assay are already positive for *Legionella* spp.

The finding that the presence of *Legionella* spp. in environmental samples is associated with geographic region is in accordance with reported differences in regional LD incidence rates in The Netherlands in the 1987-2000 period (11). This confirmation urges more detailed research into the origin of this phenomenon. Possibly, regional differences in drinking water production techniques (surface water versus ground water) can explain the association. The seasonal differences in presence of *Legionella* spp. in environmental samples coincides with documented seasonal variation in LD incidence (6,11).

With the results of this study some idea can be given about the cost-effectiveness of the Dutch national source identification programme. Given an average hospital stay of 10 days and an average cost of Euro 700 per day, a prevented LD patient would save Euro 7000. Total costs of the outbreak detection programme are Euro 125,000 per year, meaning that 18 prevented LD patients each year would turn the programme cost-effective. Although information is sparse on the number of clustered cases over time that can be attributed to a single source of infection, it seems reasonable to assume that each single source would on average present five LD patients in 10 years. The identification and elimination of 17 cluster-related sources during our 2-year study period would then lead to prevention of 46 (85
minus 29 who occurred despite first control measures) LD patients in the next 8 (ten minus two) years, or almost 6 patients each year. If every 2 years 17 new cluster-related sources would continue to be identified, the programme would become cost-effective in a little over 6 years. Probably it would become cost-effective sooner, since the above calculation did not include indirect costs of morbidity and mortality nor indirect benefits resulting from the control measures implemented at all LD patient related sources as identified by public health officials. Let alone the costs of prevented outbreaks.

In conclusion, we have shown that source identification on a national scale can be successful in terms of outbreak detection and secondary prevention of LD, provided that source elimination procedures are effective. Furthermore, we have stressed the need for culture-based diagnosis of LD and made it plausible that a national source identification programme can be cost-effective on the medium long term.

ACKNOWLEDGEMENTS

We want to thank all public health physicians and nurses of the 39 regional public health services who helped identifying potential sources of infection. We also thank the hospital doctors and microbiologists for making available patient isolates for genotyping. We are grateful to the officials of the Ministry of the Environment for their assistance in source elimination. Furthermore we thank Anita Warris and Marja Kleinee at the Health Inspectorate for their kind collaboration in data collection. We thank Kim van der Zwaluw at the National Institute of Public Health and the Environment for performing and interpreting the amplified fragment length polymorphism assays. We thank Professor Roel A. Coutinho, Director of the Centre for Infectious Diseases Control, for his critical comments on the manuscript.

This study was supported by a grant of the Public Health Stimulation Fund (Fonds OGZ). This institute did not play a role in any aspect of the study or in the writing of this paper. All authors are independent from this fund.

REFERENCES

het Waterleidingbesluit en het Besluit hygiene en veiligheid badinrichtingen en zwemgelegenheden (preventie van *Legionella* in leidingwater)"


