Legionnaires’ disease in the Netherlands, 1998-2006

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Legionnaires’ disease and gardening

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\textbf{ABSTRACT}

\textit{Legionella longbeachae} was cultured from the sputum of a patient suffering from Legionnaires’ disease. Source identification efforts included analysis of samples of potting soil from the patient’s garden, and a genotypically indistinguishable strain of \textit{L. longbeachae} was cultured from this material. Following examination of a national collection of \textit{Legionella} isolates, two more patients with indistinguishable genotypes were identified. One of these patients had visited a garden centre in the same municipality in which the index patient had acquired his potting soil. The study demonstrated the value of systematic collection of identification data and patient isolates over a prolonged period.

\textbf{Key words.} Legionnaires’ disease, pneumonia, public health, environmental exposure, \textit{Legionella longbeachae}, soil.

Legionnaires’ disease (LD) is a pneumonia caused by \textit{Legionella} spp., predominantly (>90\%) \textit{Legionella pneumophila}. Worldwide, the second most commonly isolated species is \textit{Legionella longbeachae}\textsuperscript{[1]}. In Australia, this species is responsible for 30\% of reported cases of LD. The transmission of this pathogen has been associated in Australia and the USA with handling potting soil, [2,3] but to our knowledge, there have been no previous reports of such an association in Europe.

In December 2004, the Dutch National \textit{Legionella} Outbreak Detection Programme (\textit{nLODP}) was informed of a patient with LD (date of onset, 4 November 2004). \textit{L. longbeachae} was cultured from the sputum of the patient, who had died 2 weeks following admission. A recent change in the Dutch regulations, abandoning notification of LD caused by species other than \textit{L. pneumophila}, meant that the patient had not been notified to the Ministry of Health. An interview with the patient’s relatives in December 2004 revealed that the patient, a male who smoked cigarettes, aged 67 years, with no known underlying disease, had been working in his garden with commercial potting soil during the week before his illness. On the basis of this information, workers of the \textit{nLODP} collected water and biofilm samples from the patient’s home, and potting soil samples from the patient’s garden. All water and biofilm samples were

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negative, but *Legionella* spp. were cultured from the potting soil. Amplified fragment length polymorphism analysis [4] identified two distinct genotypes, one belonging to *L. pneumophila* and one to *L. longbeachae*. The DNA banding pattern of the latter was indistinguishable from that of the *L. longbeachae* isolate from the patient’s sputum (Fig. 1, lanes 1 and 4). The other isolate was typed as *L. pneumophila* serogroup 7–14.

The NLDP has access to data from all LD patients who have been notified in the Netherlands since 1987, and a collection of 352 patient-derived *Legionella* isolates. Although only five of these isolates are *L. longbeachae*, a patient with LD (date of disease onset, 7 August 2000) caused by *L. longbeachae* had visited a garden centre during his incubation period in the same community where the index patient had obtained his potting soil. The second patient, a non-smoking male aged 81 years, had no other underlying disease apart from type 2 diabetes mellitus, and had not been working in a garden or with potting soil. The DNA banding pattern of the *L. longbeachae* isolate from this patient’s sputum was indistinguishable from that of the isolate from the index patient and the potting soil (Fig. 1, lane 3). Also indistinguishable was a further *L. longbeachae* DNA pattern for an isolate from the sputum of an LD patient (date of disease onset, 27 August 2000) (Fig. 1, lane 2) who had been working with potting soil in his garden during the incubation period. This patient lived 65 km from the index patient, making it unlikely that he had used potting soil from the same garden centre. The third patient was a non-smoking male, aged 69 years, who had suffered for 5 years from chronic lymphocytic leukaemia, for which he did not use medication. The fourth and fifth *L. longbeachae* isolates from the collection both showed distinct DNA banding patterns (Fig. 1, lanes 5 and 6) and were unrelated to the three clustered patient isolates.

Unfortunately, more precise information was not available, since all three clustered patients died following hospital admission. Aspiration of small contaminated potting soil particles seems a rather exotic explanation for transmission of *L. longbeachae*[5]. Aerosol-aided spread following air movement and evaporation of water in potting soil seems more likely, as this resembles waterborne spread, in which aerosols are a vehicle that enables legionellae to escape from the liquid environment. Aerosols that can be inhaled by humans (<5 μm) evaporate in milliseconds at standard environmental air temperature and humidity. [6] This may explain why the second patient, who did not handle potting soil, still became infected.

During the last 3 years, six of the 37 clusters of LD patients identified by the NLDP have been associated with garden centres, with no satisfactory explanation. Although ten of the
12 patients involved were diagnosed with \textit{L. pneumophila} infection (four culture-positive for serogroup 1, and one culture-positive for serogroup 3), transmission involving potting soil should not be ruled out in view of the present finding of \textit{L. pneumophila} in the potting soil used by the index patient. In Australia, [2] but not in Japan, [7] \textit{L. pneumophila} has been cultured from potting soils. In addition, composted plant material should be considered as a possible source of infection for \textit{L. pneumophila}. In Australia, all composted plant material from large producers contained \textit{L. pneumophila}, although these belonged almost exclusively to serogroup 2–14. [5] More data are required to evaluate the possible relevance of this route of transmission in Europe. Two soil surveys in Europe for \textit{L. longbeachae} and \textit{L. pneumophila} have both been negative, [2,8] but the present study contradicts the earlier hypothesis that potting soil in Europe is free of \textit{Legionella} spp. because its major component is peat, whereas potting soils in Australia, Japan and the USA are composed primarily of sawdust and bark. [7] Since August 2002, systematic sampling by the NLODP of potential water sources in relation to patients with LD has not yielded \textit{L. longbeachae}, but \textit{Legionella anisa} was isolated from water sources in two of the 22 garden centres sampled.

In Europe, 38 countries collaborating in the European Working Group on \textit{Legionella} Infections have agreed to use amplified fragment length polymorphism typing for \textit{L. pneumophila} serogroup 1, [9] later followed by a more sophisticated sequence-based typing scheme. [10] To date, neither technique has been used for typing \textit{L. longbeachae}, but results with allozyme electrophoresis and restriction fragment length polymorphism analysis suggest that the \textit{L. longbeachae} isolates were closely related to each other and to most of the Australian environmental strains. [11] Macrogen restriction digestion with SfiI, followed by pulsed-field gel electrophoresis, distinguished three distinct pulsotypes with >65% similarity, and 11 subgroups with >88% similarity. [12] The amplified fragment length polymorphism assay is preferred to pulsed-field gel electrophoresis [13] by the European Working Group on \textit{Legionella} Infections for genotyping of \textit{L. pneumophila}, but the index of diversity for \textit{L. longbeachae} requires further investigation.

In conclusion, given the low incidence of LD caused by \textit{L. longbeachae}, the described cluster of isolates is remarkable. It is clear that it is worthwhile sampling potential sources of infection for LD caused by species other than \textit{L. pneumophila}. Furthermore, the results confirm the value of systematic collection of notification data and patient isolates over a prolonged period. [14]

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REFERENCES