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Presence and Persistence of Viable, Clinically Relevant *Legionella pneumophila* Bacteria in Garden Soil in the Netherlands

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ABSTRACT

Garden soils were investigated as reservoirs and potential sources of pathogenic *Legionella* bacteria. *Legionella* bacteria were detected in 22 of 177 garden soil samples (12%) by amoebal coculture. Of these 22 *Legionella*-positive soil samples, seven contained *Legionella pneumophila*. Several other species were found, including the pathogenic *Legionella longbeachae* (4 gardens) and *Legionella sainthelensi* (9 gardens). The *L. pneumophila* isolates comprised 15 different sequence types (STs), and eight of these STs were previously isolated from patients according to the European Working Group for *Legionella* Infections (EWGLI) database. Six gardens that were found to be positive for *L. pneumophila* were resampled after several months, and in three gardens, *L. pneumophila* was again isolated. One of these gardens was resampled four times throughout the year and was found to be positive for *L. pneumophila* on all occasions.

IMPORTANCE

Tracking the source of infection for sporadic cases of Legionnaires' disease (LD) has proven to be hard. *L. pneumophila* ST47, the sequence type that is most frequently isolated from LD patients in the Netherlands, is rarely found in potential environmental sources. As *L. pneumophila* ST47 was previously isolated from a garden soil sample during an outbreak investigation, garden soils were investigated as reservoirs and potential sources of pathogenic *Legionella* bacteria. The detection of viable, clinically relevant *Legionella* strains indicates that garden soil is a potential source of *Legionella* bacteria, and future research should assess the public health implication of the presence of *L. pneumophila* in garden soil.

*L*egionella is an opportunistic pathogen that can cause legionellosis (1). Legionellosis refers to two distinct clinical syndromes: Legionnaires' disease (LD) and Pontiac fever. The vast majority of the clinical isolates in Europe (2–4) and the United States (5) constitute *Legionella pneumophila* serogroup 1 (SG1). In Australia, New Zealand, and Thailand, *Legionella longbeachae* is an important cause of disease (6–8). *Legionella* bacteria are ubiquitous in natural matrices and manmade water systems (9). Some of these systems and matrices can act as sources of *Legionella* infection and include cooling towers (10), whirlpools (11), thermal springs (12), and wastewater treatment plants (13). Potting soil is also a source of *L. longbeachae* infection (14, 15).

In source investigations, alongside epidemiological evidence, a genotypic match between environmental and clinical strains is needed to identify the source of infection. Tracking the source of infection for sporadic cases of LD has proven to be hard (16). Studies in the Netherlands, England, and Wales showed that common clinical *L. pneumophila* strains are only rarely found in the environment (17, 18). In these studies, *L. pneumophila* genotypes isolated from patients were compared with genotypes isolated from the environment. The environmental sources of *Legionella* in these studies comprised manmade water systems (domestic water distribution systems, cooling towers, and spa pools).

L. pneumophila sequence type 47 (ST47) is the ST that is most frequently isolated from patients in the Netherlands (26.9% of clinical isolates available for typing) (16). Strikingly, since the start of the National *Legionella* Outbreak Detection Programme in the Netherlands in 2002, this ST was only found three times in the environment during outbreak investigations, which concerned outdoor whirlpools that were involved in two combined out-

breaks of LD and Pontiac fever and one solitary case of LD. As all three whirlpools were located outside, it was hypothesized that the outdoor environment was an influence, and after further investigation, *L. pneumophila* ST47 was isolated from a soil sample from the garden of the most recent outbreak (19). It was suggested that the ST47 strain was transmitted from garden soil to the whirlpool by wind or by people entering the whirlpool with soil on their feet.

It is possible that garden soil plays a role in *Legionella* infection. Although potting soil is a well-studied reservoir and known source of *Legionella*, not much is known about *Legionella* in garden soil. Hughes and Steele (20) showed the presence of *Legionella* in six garden soils that were mixed with composted materials. Furthermore, there is evidence that natural soil is a reservoir and source of *Legionella*. Wallis and Robinson (21) reported on a LD patient that had worked at a plant nursery in the week prior to illness. A *L. pneumophila* strain with an indistinguishable genotypic profile,

determined by pulsed-field gel electrophoresis (PFGE), was isolated from a field in which the patient had spent time potting plants. In several other studies *L. pneumophila* strains have been isolated from natural soil (22–25). In two of these studies, some of the obtained sequence types (STs) had previously been detected in cases of LD (22, 23).

The aim of the current study was to investigate garden soil as a reservoir of viable, clinically relevant *Legionella* bacteria. Garden soils were sampled throughout the year. Furthermore, we studied whether *L. pneumophila* could persist in soil over time by resampling *Legionella*-positive gardens after several months. An amoebal coculture method was applied to detect *Legionella* bacteria in the soil. Amoebal coculture has been proven successful for the isolation of *Legionella* bacteria in samples, such as soil, with a lot of background flora (26, 27).

MATERIALS AND METHODS

Sampling of garden soils. Garden soil samples were collected over a period of 1 year (February 2014 to January 2015). In order to obtain a large and diverse number of samples, colleagues from the National Institute for Public Health and the Environment and from the Regional Public Health Laboratory Kennemerland were asked to supply soil samples from their own gardens and/or the gardens of friends and family. Samples were to consist of soil from a soil bed at any place within their own garden, irrespective of type of garden, cultivation, or planting. Sterile spoons and jars were provided and were accompanied by an instruction form and short questionnaire. Participants were asked to sample the upper 2 cm of soil at one place in the garden. Upon arrival, the samples were stored at 4°C until analysis. If *L. pneumophila* was isolated from a garden, then the owners were asked to resample their garden after several months at the same location as the first sampling.

Pretreatment of the samples. Prior to analyses, 5 g of each soil sample was resuspended in 5 ml of sterile distilled water. These suspensions were vortexed for approximately 10 s and incubated at room temperature for 1 h. The soil suspensions were vortexed again just before amoebal coculture.

Amoebal coculture method. The amoebal coculture method for the detection of *Legionella* was performed as described previously (27). Briefly, *Acanthamoeba castellanii* ATCC 30234 (American Type Culture Collection, Manassas, VA, USA) was grown in 75-cm^2 culture flasks (Corning Inc., New York, NY, USA) with 15 ml of peptone-yeast extract-glucose (PYG) broth at 25°C. Prior to the infection, the PYG broth was removed, and the amoebae were resuspended in 15 ml Page's amoebal saline (PAS) (28). The amoebae suspension was centrifuged at 850 × *g* for 10 min, and the pellet was subsequently resuspended in 15 ml PAS. This washing step was repeated 2 times. Cells were seeded in a 12-well microplate (Corning) at a density of 5 × 10^5 cells/ml of PAS. In each well, 1 ml of PAS with amoebae was inoculated with 100 μl of sample, and each sample was tested in triplicate (three wells). Thus, amoebae were inoculated with a total of 300 μl of the suspension. By testing 300 μl of the soil suspension, 0.3 g of the soil sample was tested. The theoretical detection limit was therefore 3.3 CFU/g soil.

The amoebal plates were incubated at 32°C. As a negative control, one well with amoebae was not inoculated with a sample. After 3 days of incubation, 100 μl of each suspension was subcultured on a new plate with freshly seeded amoebae. After another 3 days of incubation at 32°C, 100 μl of each well was serially diluted 10-fold in PAS. Of the 10^4-, 10^5-, 10^6-, 10^7-, and 10^8-fold dilutions, 100 μl was plated on buffered charcoal yeast extract (BCYE) plates (Oxoid Ltd., Hampshire, United Kingdom). The 10^5- and 10^6-fold dilutions were also cultured on glycine-vancomycin-polymyxin B-cycloheximide (GVPC) plates (Oxoid Ltd., Hampshire, United Kingdom). After 4 and 7 days of incubation at 37°C, the BCYE and GVPC plates were inspected for *Legionella*-like colonies with a stereo microscope (magnification, 40×; Olympus).

Confirmation and typing of *Legionella* isolates. Suspected *Legionella* colonies were tested for their inability to grow on BCYE medium without cysteine (Oxoid Ltd., Hampshire, United Kingdom). Strains that were unable to grow on the medium without cysteine were further subtyped by polyclonal antisera (*L. pneumophila* SG1, SG2 to SG14, and *Legionella* spp.) coupled to latex beads (*Legionella* latex test; Oxoid Limited, Hampshire, England) and subsequently stored at −70°C. The strains were later identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). Strains that could not be recultured prior to MALDI-TOF MS identification and that showed no clear agglutination response in the latex test were confirmed with a *Legionella* species-specific PCR targeting the 5S rRNA gene (29). *L. pneumophila* isolates were genotyped by the standard sequence-based typing (SBT) method of the European Working Group for *Legionella* Infections (EWGLI) using seven genes (*flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, and *neuA*) (30, 31). The SBT profiles were generated using the high-throughput multilocus sequence typing (HiMLST) method that employs next-generation sequencing (32). *L. pneumophila* SG1 isolates were also subtyped by monoclonal antibody (MAb) subgrouping using the Dresden MAb panel (33).

Statistical analysis. Univariate and multivariate logistic regressions were performed as described by Hosmer and Lemeshow (34). Univariate analysis was used to identify potential associations between the positivity of gardens for *Legionella* and questionnaire and weather variables. The weather variables, i.e., precipitation and ambient temperature related to sampling days, were obtained from the Royal Netherlands Meteorological Institute (KNMI). For each garden, data were selected from the weather station closest to that garden. Continuous variables (garden age and weather characteristics) were categorized into classes of approximately equal size. Variables with a univariate *P* value of ≤0.25 (two-tailed likelihood ratio test) were selected for multivariate logistic regression. Prior to the multivariate analysis, the correlation coefficients of all variable combinations were determined, where a correlation coefficient of 0.25 or lower was considered noninterfering in the multivariate analysis. Subsequently, backward elimination was used until the remaining variables exhibited a significant association with a *P* value of <0.05 (two-tailed likelihood-ratio test). The statistical analyses were performed using SAS software v.9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Isolation of *Legionella* spp. and *L. pneumophila* from garden soils. Over 12 months, a total of 177 unique gardens were sampled. Per month, between 10 and 20 samples were submitted by our colleagues, with an average of 15 (see Table 1). In total, 22 of 177 samples (12%) were positive for *Legionella* spp. Out of these 22 positive samples, seven (32%) contained *L. pneumophila* (garden samples 1, 4, 5, 8, 13, 18, and 22; see Table 2). Both *L. pneumophila* SG1 and non-SG1 isolates were obtained, and 15 different STs were identified. In five of these samples, other *Legionella* species were also detected besides *L. pneumophila*, namely, *L. longbeachae* (4 samples), *L. sainthelensi* (2 samples), *Legionella bozemanii* (1 sample), and *Legionella feeleii* (1 sample). In the remaining 15 samples, several *Legionella* species were detected (Table 2), including *L. sainthelensi* (7 samples), *L. feeleii* (2 samples), *L. bozemanii* (1 sample), *Legionella cincinnatiensis* (1 sample), *Legionella anisa* (1 sample), and *Legionella wadsworthii* (1 sample). For 3 of the 15 samples, *Legionella* could not be further typed by MALDI-TOF MS because the bacteria could not be recultured.

Positive samples were found throughout the year (see Table 1). The ambient mean daily temperatures on sampling days ranged from −1.9°C to 22.8°C (35). Positive garden soil samples were taken on days with ambient mean temperatures ranging between

TABLE 1 Numbers of *Legionella* species-positive and *L. pneumophila*-positive garden soil samples per month[a]

Sampling yr	Sampling mo	Mean temp (°C) (range)	Total precipitation (mm)	No. of samples analyzed	No. of *Legionella* species-positive samples (no. of *L. pneumophila*-positive samples)
2014	February	6.5 (3.9–10)	66.4	14	2 (1)
	March	8.4 (4.5–14.7)	25.7	16	1 (0)
	April	12.1 (7.2–17.1)	58.4	16	4 (2)
	May	13.2 (7.9–19.9)	102.0	15	0 (0)
	June	16.2 (13–21.6)	30.3	16	2 (1)
	July	19.8 (15–26.4)	137.1	20	3 (0)
	August	16.1 (12.4–20.5)	149.0	10	2 (1)
	September	15.9 (21.6–19.3)	20.5	16	3 (0)
	October	13.4 (9.9–17.3)	74.9	16	0 (0)
	November	8.2 (2.5–14.8)	46.8	15	2 (1)
	December	4.8 (−1.2–11.5)	99.5	11	2 (0)
2015	January	4.0 (−2.8–9.9)	115.7	12	1 (1)
Total				177	22 (7)

[a] The mean ambient temperature (°C) per sampling month and the total precipitation (mm) per month are indicated (35).

0.7°C and 21.5°C. The precipitation sums of the 14 days preceding the sampling day varied between 0 and 113 mm for all sampling days and between 2 and 81 mm for the sampling days at which *Legionella* was detected.

Resampling *L. pneumophila*-positive garden soils. Six of the seven *L. pneumophila*-positive gardens were resampled after several months, and four again tested positive for *Legionella* (gardens 1, 4, 13, and 18; see Table 3). One of the *L. pneumophila*-positive gardens was not resampled because this garden was found to be positive at the end of the project. For three of the four gardens, *L. pneumophila* was again isolated in the second sampling (gardens 1, 13, 18). Garden 1 was resampled four times throughout the year and was found to be positive for *L. pneumophila* on all occasions. Several *L. pneumophila* STs were detected, with ST477 present on all four occasions and ST710 and ST84 present on three out of four occasions. *L. longbeachae* was also isolated on all 4 samplings of garden 1.

Clinical relevance of *L. pneumophila* isolates. A total of 70 *L. pneumophila* isolates were typed by SBT. For five isolates, a ST could not be retrieved due to the failure of amplification of one or more gene targets. The remaining 65 isolates were classified into 15 different STs (see Table 4). According to the EWGLI SBT database (36) (accessed on 17 February 2016), eight of these STs (ST84, ST115, ST462, ST465, ST477, ST710, ST863, and ST1856) were previously isolated from patients. Some STs were found regularly in garden soils, namely, ST84, ST115, ST477, and ST710. ST84 was detected most often; of the seven *L. pneumophila*-positive gardens, ST84 was found in all but one. Of the *L. pneumophila* SG1 isolates, 16 were MAb 3/1 positive and 10 were MAb 3/1 negative (see Table S1 in the supplemental material, which shows all of the typing data of the *L. pneumophila* garden isolates).

Univariate and multivariate analyses. The questionnaire covered the following variables: characteristics of the garden (type, size, location, age), use of potting soil/compost, origin of used potting soil/compost, use of other fertilizers (e.g., manure, artificial fertilizer), use of pesticides/herbicides, use of tap water for watering, presence of an outdoor whirlpool, season of sampling, and frequency of gardening in gardening season (see Table S2 in the supplemental material). The owners of all 177 sampled gardens filled in the questionnaire. In addition, eight weather variables were analyzed, namely, precipitation sum on the sam-

pling day, precipitation sum in the 14 days preceding the sampling day, ambient temperature (minimum, mean, maximum) on the sampling day, and ambient temperature (minimum, mean, maximum) in the 14 days preceding the sampling day.

Six of the examined variables reached a *P* value of ≤0.25 in the univariate logistic regression and were analyzed in the multivariate analysis, i.e., garden size, garden age, use of potting soil/compost, use of other fertilizers, precipitation sum on the sampling day, and mean temperature in the 14 days preceding the sampling day (see Table S2 in the supplemental material). One variable (surrounding area) had a prevalence of zero in two out of three categories and could not be analyzed in the multivariate model. Correlations between all variables appeared to be low (i.e., $r ≤ 0.25$). Multivariate logistic regression analysis identified none of the variables to be statistically significantly associated with the presence of *Legionella* in the gardens.

DISCUSSION

Viable *Legionella* strains, including *L. pneumophila*, were isolated by amoebal coculture from 12% of 177 investigated garden soils in the Netherlands, indicating that garden soil is a reservoir of *Legionella* bacteria. The majority of the isolated *L. pneumophila* SG1 strains were found to be MAb 3/1 positive. MAb 3/1 positivity is considered an indication of virulence since this monoclonal antibody recognizes a virulence-associated epitope (33). Eight STs found in garden soils were clinically relevant according to the EWGLI SBT database (36). However, the STs that are most often detected in patients in the Netherlands (ST1, ST47, and ST62) (16) were not detected in garden soils. Of the 15 detected STs in this study, three were also found in patients in the Netherlands (ST84, ST115, and ST477). However, these STs are relatively uncommon; they were found in three patients, four patients, and one patient, respectively (since 2005). Strikingly however, these three clinically relevant STs belonged to the most frequently isolated STs in garden samples (see Table 4). It is possible that some conditions in garden soils are favorable for the growth of these clinically relevant STs. So, although garden soil is probably not the source of most infections with *L. pneumophila* in the Netherlands, it should be taken into account that it

TABLE 2 Detected *Legionella* species in garden soil samples

Sampling mo and yr	Garden soil sample	*Legionella* spp.[a]	*L. pneumophila* serogroup(s) (no. colonies analyzed)	Sequence type(s)
February 2014	1	*L. pneumophila*	1 (9)	84, 477, 863
		L. pneumophila	2–14 (2)	465, 710
		L. longbeachae	NA[b]	NA
	2	*L. sainthelensi*	NA	NA
March 2014	3	*L. cincinnatiensis*	NA	NA
April 2014	4	*L. pneumophila*	1 (6)	84, 115, 477, 2028, 2032
		L. pneumophila	2–14 (12)	863, X[c]
		L. bozemanii	NA	NA
		L. feeleii	NA	NA
	5	*L. pneumophila*	2–14 (8)	2025, 2026, X
	6	*L. wadsworthii*	NA	NA
	7	*L. sainthelensi*	NA	NA
June 2014	8	*L. pneumophila*	1 (2)	84, 710
		L. pneumophila	2–14 (1)	462
		L. longbeachae	NA	NA
	9	*L. sainthelensi*	NA	NA
July 2014	10	*L. feeleii*	NA	NA
	11	*L. sainthelensi*	NA	NA
	12	*Legionella* spp.[d]	NA	NA
August 2014	13	*L. pneumophila*	2–14 (4)	710
		L. longbeachae	NA	NA
		L. sainthelensi	NA	NA
	14	*L. sainthelensi*	NA	NA
September 2014	15	*L. sainthelensi*	NA	NA
	16	*Legionella* spp.[d]	NA	NA
	17	*Legionella* spp.[d]	NA	NA
November 2014	18	*L. pneumophila*	1 (15)	84, 477, 1856, 2022, 2029
		L. pneumophila	2–14 (3)	84, 115, 710
		L. longbeachae	NA	NA
		L. sainthelensi	NA	NA
	19	*L. feeleii*	NA	NA
		L. anisa	NA	NA
December 2014	20	*L. sainthelensi*	NA	NA
	21	*L. bozemanii*	NA	NA
January 2015	22	*L. pneumophila*	1 (2)	84

[a] Species were typed by MALDI-TOF MS.
[b] NA, not applicable.
[c] X, a sequence type could not be retrieved due to failure of amplification of one or more gene targets.
[d] *Legionella* spp. were not further typed because the bacteria could not be recultured.

may play a role in *Legionella* transmission to humans, which warrants further investigation.

L. sainthelensi was found most often in garden soil; it was isolated from 10 of the 22 positive gardens (see Tables 2 and 3). *L. sainthelensi* can be infectious to humans (37, 38) and was first isolated in the United States from fresh water (39). *L. pneumophila* was the second most isolated species, and *L. longbeachae* was the third. Strikingly, *L. longbeachae* was always detected in combination with *L. pneumophila*. *Legionella* bacteria were detected in garden soils throughout the year. No association was found between

Legionella-positive gardens and temperature or precipitation. Evidence was found that *L. pneumophila* can persist in garden soil. For three gardens, *L. pneumophila* was detected again after 4 to 7 months, and for one garden, *L. pneumophila* was found on four sampling occasions over a period of 1 year.

Interestingly, *Legionella* was absent from garden soils in rural areas, whereas 14.6% of garden samples in urban areas were positive for *Legionella* (see Table S2 in the supplemental material, variable "surrounding area"). However, since only 9 gardens in rural areas were investigated, no firm conclusion can be drawn about the differences in *Legionella* presence in rural versus urban areas. *Legionella* was also absent from gardens in mixed areas (gardens located at the edge of a village, adjacent to a rural area).

It is possible that *Legionella* in garden soil originates from compost or potting soil. One Australian study, by Hughes and Steele (20), showed the presence of *Legionella* in six garden soils, of which five contained *L. pneumophila*. These soils were mixed with composted materials that were found to contain *Legionella*. Compost (20, 40, 41) and potting soil (42–44) are reservoirs of *Legionella*, and it is possible that *Legionella* is introduced into garden soil by the use of compost or potting soil in the garden. To investigate this possibility, genotypes isolated from garden soils can be compared to genotypes isolated from composts and potting soils. In this study, compost or potting soil samples were provided by the garden owners when available, but they were only analyzed when the compost/potting soil was ever applied to the area where the soil sample was taken and when the soil sample was found to be positive for *L. pneumophila*. For the seven *L. pneumophila*-positive gardens, only one potting soil sample was analyzed but not found to be positive. Furthermore, no association was found between *Legionella*-positive gardens and the reported use of potting soil or compost. We found only one publication that used SBT to type two *L. pneumophila* strains that were isolated from composted materials (40). Interestingly, these were ST84, the most frequently isolated ST in this study. In contrast to the limited number of garden soil studies, several studies have investigated natural soil as a reservoir of *L. pneumophila* (22–24, 27). No ST similarities were observed between the reported STs in these studies and the current study.

A limitation of the use of an amoebal coculture method for the isolation of *Legionella* is that selectivity for certain *Legionella* strains might be introduced. It is possible that certain STs replicate better in *A. castellanii* than other STs, resulting in an outcome that is not representative of the natural situation. However, selectivity was not shown in a previous study in which a batch of 23 different *L. pneumophila* strains was tested (26). The batch was comprised of different monoclonal subtypes, both positive and negative for MAb 3/1, and 16 different STs. These strains, including the clinically relevant ST1 and ST47, were all shown to replicate similarly in amoebal coculture with *A. castellanii*. Another drawback of the amoebal coculture method is its limited sensitivity since only a small amount of soil is investigated per sample. However, for soil samples, amoebal coculture seems to be the best method in order to obtain isolates. In previous studies, we have shown that for samples containing many other bacteria, like soil, amoebal coculture has a higher positivity rate than culture techniques that are based solely on agar plates (26, 27). Another limitation of amoebal coculture is that only a restricted number of samples can be investigated since it is a rather laborious method. Therefore, only one

TABLE 3 Persistence of *Legionella* species in gardens 1, 4, 13, and 19, sampled on 2 to 4 occasions

Garden	Sampling parameters	First sampling	Second sampling	Third sampling	Fourth sampling
1	Sampling date (mo, yr)	February 2014	September 2014	November 2014	February 2015
	Detected species	*L. pneumophila* SG1	*L. pneumophila* SG1	*L. pneumophila* SG1	*L. pneumophila* SG1
		L. pneumophila SG2–14	*L. pneumophila* SG2–14	*L. longbeachae*	*L. pneumophila* SG2–14
		L. longbeachae	*L. longbeachae*		*L. longbeachae*
	Sequence types	84, 465, 477, 710, 863	84, 477, 710	84, 115, 477	477, 710
4	Sampling date (mo, yr)	April 2014	September 2014		
	Detected species	*L. pneumophila* SG1	*L. feeleii*		
		L. pneumophila SG2–14	*L. sainthelensi*		
		L. bozemanii			
		L. feeleii			
	Sequence type(s)	84, 115, 477, 863, 2028, 2032, X[a]	NA[b]		
13	Sampling date (mo, yr)	August 2014	January 2015		
	Detected species	*L. pneumophila* SG2–14	*L. pneumophila* SG1		
		L. longbeachae	*L. pneumophila* SG2–14		
		L. sainthelensi	*L. bozemanii*		
			L. dumoffi		
	Sequence type(s)	710	84, 115, 710, 2080, X		
18	Sampling date (mo, yr)	November 2014	March 2015		
	Detected species	*L. pneumophila* SG1	*L. pneumophila* SG1		
		L. pneumophila SG2–14	*L. longbeachae*		
		L. longbeachae	*L. feeleii*		
		L. sainthelensi			
	Sequence types	84, 115, 477, 710, 1856, 2022, 2029	2022, X		

[a] X, a sequence type could not be retrieved due to failure of amplification of one or more gene targets.
[b] NA, not applicable.

sample per garden was analyzed, while multiple samples would probably have influenced the positivity rate. A more thorough investigation may have been done using molecular techniques, like PCR. However, molecular methods do not render isolates, which are necessary for typing purposes. Due to these limitations, including low sensitivity and the restricted number of samples, an accurate estimate of the true prevalence of *Legionella* in garden soils cannot be made. In order to study the preva-

TABLE 4 Isolated sequence types from garden soils and clinical relevance of the sequence types according to the EWGLI SBT database[a]

Sequence type	No. of gardens[b]	No. of patients[c]
84	6	13
115	4	8
710	4	2
477	3	4
863	2	1
462	1	1
465	1	2
1856	1	1
2022	1	0
2025	1	0
2026	1	0
2028	1	0
2029	1	0
2032	1	0
2080	1	0

[a] The database was accessed on 17 February 2016 (36).
[b] Number of gardens from which the sequence type was isolated.
[c] Number of patients from which the sequence type was isolated according to the EWGLI SBT database. A value of 0 indicates that no clinical strains were reported.

lence, another sampling strategy and other analysis methods should be chosen.

The significance of the presence of *Legionella* in garden soils to public health is not clear. No patients are known to have been infected by *Legionella* originating from garden soil. In several older publications, natural soil was considered to be a possible source of infection (25, 45–49) because excavation sites were believed to be associated with LD cases (50, 51). However, these studies provided no or little evidence for soil as a source of *Legionella* (9). One more recent study provided evidence for infection caused by *L. pneumophila* originating from natural soil (21). The infective strain was isolated from a field where the LD case had spent several days potting plants.

In conclusion, garden soil is a reservoir of *L. pneumophila* and *Legionella* spp. and may be an alternative source of *Legionella* that is not considered in source investigations, especially for some soil-specific strains like ST84, ST115, and ST477. Whether the presence of *Legionella* in garden soil has an impact on public health is not clear; no cases are linked to *Legionella* in garden soil, and none of the most prevalent Dutch clinical strains were identified in garden soil in this study. A case-control study may reveal whether gardening or working with garden soil is a risk factor for contracting LD, warranting targeted interventions. Prevalence should be studied in more detail, and *Legionella* concentrations in garden soil should be determined. Furthermore, it should be investigated how soil and other environmental conditions, i.e., weather characteristics, influence viability, growth, and virulence of *Legionella* in garden soil. Furthermore, it is important to investigate if and how *Legionella* bacteria can aerosolize from soil and which gardening activities might pose a risk.

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