ORIGINAL ARTICLE 10.1111/j.1469-0691.2009.02742.x

Commercial potting soils as an alternative infection source of *Legionella pneumophila* and other *Legionella* species in Switzerland

S. Casati, A. Gioria-Martinoni and V. Gaia

Swiss National Reference Centre for Legionella, Cantonal Institute of Microbiology, Bellinzona, Switzerland

Abstract

Legionella spp. are pathogens that can cause Legionnaires' disease in humans through inhalation of contaminated aerosols. The principal reservoir for these microorganisms is water, but *Legionella* spp. have been isolated from composted vegetable and plant material, and from many potting mixes as well. In Australia, there have been several cases of Legionnaires' disease in which *Legionella longbeachae* has been isolated from potting soils. In Switzerland, the source of infection cannot always be identified as water or cooling towers: therefore, we have investigated 46 commercially available potting soils in Switzerland to determine the presence of *Legionella* spp. We were able to detect *Legionella* spp. in 45.7% (21/46) of the potting soil samples analysed by culture. *Legionella pneumophila* was present in 19.6% (9/46) of the samples and *L. pneumophila* serogroup 1 in 6.5% (3/46). Quantification by both culture and quantitative real-time PCR revealed high concentrations of legionellae in potting soils, ranging between 10^3 CFU/g and 10^5 CFU/g and 10^4 genomic units (GU)/g and 10^6 GU/g, respectively. Thus, potting soils may represent an alternative reservoir for *Legionella* spp. in Switzerland.

Keywords: Aerosol, disease reservoir, *Legionella*, potting soils, transmission
Original Submission: 16 May 2008; **Revised Submission**: 18 August 2008; **Accepted**: 29 September 2008
Editor: G. Greub
Clin Microbiol Infect 2009; **15:** 571–575

Corresponding author and reprint requests: V. Gaia, Swiss National Reference Centre for Legionella, Cantonal Institute of Microbiology, Via Mirasole 22A, 6500 Bellinzona, Switzerland
E-mail: valeria.gaia@ti.ch

Introduction

Legionella spp. are Gram-negative bacteria that may cause Legionnaires' disease (LD), a pulmonary infection in humans, after inhalation of contaminated aerosols. The principal reservoir of these microorganisms is water, but *Legionella* spp. have also been isolated from composted vegetable and plant material as well as from potting mixes in Australia [1–3].

The LD outbreaks reported in Europe have been associated with airborne transmission of legionellae from cooling towers, showers, and other devices that produce aerosols. Towards the end of the 1980s, a significant number of sporadic infections in Australia associated with potting mixes were reported [1].

Since then, several LD cases have been identified every year in Australia, with *Legionella longbeachae* being isolated from potting soils. Recently, *Legionella pneumophila* serogroup 1 isolated from soil was related to a clinical case [4]. The study by Steele *et al.* [2] showed that *L. longbeachae* and other *Legionella* spp. could be isolated from as many as 73% of potting soils produced in Australia.

They could not be detected, however, in potting soils from Greece, Switzerland, and the UK. For a long time, therefore, contamination of soil by legionellae was considered to be limited to Australia. However, an association between LD cases and gardening or use of potting mixes has also been seen in Japan, in the USA, and, more recently, in The Netherlands [5–7]. Therefore, it would be expected that human infection in other parts of the world might occur not only after the aerosolization of contaminated water, but also after contact with contaminated potting soil.

Water and cooling towers cannot always be considered the source of infections and disease outbreaks in Switzerland. Therefore, alternative ecosystems that could be the reservoir for *Legionella* spp. have been investigated in this study. Different commercial soil mixtures were analysed using culture methods and quantitative real-time PCR (qPCR) to evaluate whether potting soils may represent an additional source of human infections in Switzerland.

Materials and Methods

We investigated the presence of *Legionella* spp. in 46 commercially available potting soils in Switzerland (packed in 3–30-L bags, and produced by the most important Swiss manufacturers) collected during the gardening season in 2006 and 2007. These samples were randomly chosen in the better-known supermarkets in all parts of the country. Two-thirds of the samples contained principally peat moss and, variably, bark, soil, soil originating from waste composting, and mineral fertilizer. The culture analysis was performed by using buffered charcoal yeast extract agar (BCYE) (Biomérieux, Geneva, Switzerland), and the two selective media modified Wadowsky-Yee (MWY) (Oxoid, Pratteln, Switzerland) and glycine–vancomycin–polymyxin B–cycloheximide (GVPC) (Biomérieux). In addition, the samples were analysed by qPCR (GeneSystems, Bruz, France). The isolation of *Legionella* spp. from potting soil samples was performed as previously described, with a few modifications [1–3]. Briefly, 5 g of each potting soil was suspended in 15 mL of sterile water. After homogenization and incubation for 30 min at room temperature, the suspensions were treated with 0.2 M HCl–KCl acid buffer (pH 2.2) to reduce the number of contaminating soil organisms. Dilutions of 1 : 100 (A) and 1 : 20 (B) were prepared from the acid-treated suspensions, and vortexed occasionally for 15 min (A) and for 10 min (B) at room temperature during incubation to disperse the sample. Then, 50-μL amounts of each acid-treated sample were plated on both non-selective and selective media, and incubated at 35 ± 1°C for 5 days. Depending on which dilution procedure was used, the amount of sample inoculated on the plate was 1/6000 or 1/30 000 of the original soil sample (5 g).

The detection limit (one colony on a plate) of the different dilutions correspond to 1200 CFU or 6000 CFU per gram of soil, respectively. *Legionella*-like colonies, one for each morphotype present on the plate, were tested for their ability to grow on media with and without cysteine. Strains unable to grow on substrates lacking cysteine were considered to be probable *Legionella* strains and further identified. Potential *L. pneumophila* strains were tested by slide agglutination (Latex test; Biomérieux) to determine the serogroup (serogroup 1 or serogroups 2–15), and then further subgrouped by immunofluorescent antibody typing, using the monoclonal antibodies from the Dresden panel [8]. Those strains considered to be *Legionella* spp. from their colony morphology were identified by sequencing of the *mip* gene [9,10], and the sequence was compared with the *mip* database available on the website of the European Working Group on *Legionella* Infections (http://www.ewgli.org). The detection limit for culture of *Legionella* spp. was 10^3 CFU per gram.

For qPCR, DNA was extracted from the acid-treated samples A by using the Gene Extract platform (GeneSystems), and subsequently amplified and quantified using the GeneDisc Cycler platform (GeneSystems), according to the manufacturer's instructions. The PCR system can quantify the presence of *L. pneumophila* (GeneDisc *L. pneumophila*) and *Legionella* spp. (GeneDisc *Legionella* species) separately. The qPCR detection limit was calculated as 10^4 genomic units (GU) per gram.

Results

Legionella strains were cultured from 21 (45.7%) of the 46 commercial potting soils analysed. The degree of contamination of the soils ranged from 10^3 CFU/g to 10^5 CFU/g. The isolates belonged to 13 *Legionella* spp.. *Legionella bozemanii* was recovered from 12 samples (26.1%), *L. pneumophila* 2–15 (serogroups 3, 6, and 10) from nine samples (19.6%), *Legionella sainthelensi* from six samples (13.0%), *Legionella micdadei* from four samples (8.7%), *L. pneumophila* 1 (Mabs subgroup Philadelphia, France/Allentown, Benidorm) from three samples (6.5%), *L. longbeachae* from two samples (4.3%), and *Legionella nautarum*, *Legionella feelei*, *Legionella birminghamensis*, *Legionella jamestowniensis*, *Legionella quinlivanii* and two unidentified strains from one sample each (Table 1). On the basis of the *mip* identification scheme, the unidentified strains showed 91% and 93% homology, respectively, with strain 81-029 and species B-IMVS-86 described by Ratcliff *et al.* [9].

Twenty samples (43.5%) contained species of *Legionella* considered to be pathogenic to humans, i.e. *L. pneumophila*, *L. bozemanii*, *L. longbeachae*, *L. micdadei*, *L. sainthelensi*, *L. feelei*, and *L. birminghamensis*. In particular, *L. pneumophila* was present in nine samples (19.6%), and in three of these (6.5%) was of serogroup 1.

The qPCR method allowed the detection of *Legionella* spp. in 41 potting soil samples (89.1%; Tables 1 and 2), whereas *L. pneumophila* was detected in 16 samples (34.8%) (Table 1). Only five samples (10.9%) were negative by qPCR (Tables 1 and 2). In the positive samples, the concentrations ranged from 10^4 GU/g to 10^6 GU/g.

Culture and qPCR methods (Table 2) showed a correspondence in 19 samples (41.3%). Two samples, from which *L. micdadei* was isolated, were positive only by culture methods. The qPCR detected *Legionella* spp. in 22 samples (47.8%) for which cultures were negative. Three samples (6.5%) were negative with both methods.

TABLE 1. Isolation by culture and quantification by culture and by quantitative real-time PCR of *Legionella* spp. from 46 potting soils available in Switzerland

Sample no.	*Legionella* spp. isolated	*Legionella* quantification			
		Culture Lp (CFU/g)	PCR Lp (GU/g)	Culture Lsp (CFU/g)	PCR Lsp (GU/g)
1	*L. bozemanii*, *L. longbeachae*, strain 81-029	–	–	220 800	119 880
2	*L. micdadei*	–	–	12 000	–
3	Lp 1 (France/Allentown), Lp 3, Lp 10, *L. jamestowniensis*	24 000	12 488	104 400	233 932
4	–	–	–	–	56 610
5	Lp 2–15, *L. bozemanii*	4800	–	70 800	147 352
6	–	–	–	–	49 117
7	–	–	–	–	–
8	–	–	–	–	32 467
9	–	–	–	–	58 275
10	Lp 10	1200	Presence[a]	–	19 980
11	–	–	–	–	51 615
12	–	–	–	–	39 127
13	–	–	–	–	100 732
14	*L. sainthelensi*	–	–	1200	84 082
15	–	–	–	–	44 122
16	*L. bozemanii*, *L. longbeachae*, *L. micdadei* species B-IMVS-86, *L. quinlivanii*	–	13 320	264 000	308 857
17	–	–	Presence[a]	–	47 787
18	–	–	–	–	10 822
19	–	–	–	–	85 747
20	–	–	–	–	78 255
21	–	–	–	–	29 137
22	–	–	–	–	65 767
23	–	–	–	–	41 625
24	*L. micdadei*	–	–	3600	–
25	*L. sainthelensi*	–	–	3600	288 045
26	–	–	22 477	–	1 660 837
27	*L. bozemanii*, *L. nautarum*	–	–	2400	218 947
28	–	–	–	–	316 350
29	*L. bozemanii*	–	21 190	6000	799 200
30	*L. feelei*, *L. londiniensis*	–	38 345	12 000	894 054
31	Lp 1 (Benidorm), Lp 6, *L. bozemanii*	26 400	29 263	56 400	513 930
32	Lp 1 (Philadelphia), Lp 10, *L. bozemanii*, *L. birminghamensis*, *L. micdadei*	27 600	72 654	46 800	806 263
33	–	–	–	–	1 879 936
34	Lp 6, *L. bozemanii*, *L. sainthelensi*	1200	44 400	8400	268 418
35	*L. bozemanii*, *L. sainthelensi*	–	35 318	13 200	187 690
36	Lp 2–15, *L. bozemanii*, *L. sainthelensi*	3600	23 209	36 000	2 916 272
37	Lp 6, *L. sainthelensi*	3600	41 372	4800	789 109
38	*L. bozemanii*	–	31 281	60 000	1 705 363
39	–	–	–	–	847 636
40	–	–	–	–	62 563
41	–	–	–	–	345 109
42	–	–	21 190	–	889 009
43	–	–	–	–	–
44	*L. bozemanii*	–	–	6000	85 756
45	–	–	–	–	–
46	Lp 2–15	6000	21 190	6000	903 456

–, negative result; Lp 2–15, serogroup not determined; Lp, *Legionella pneumophila*; Lsp, *Legionella* spp.
[a]Detection of DNA under the quantitation limit.

TABLE 2. Comparison of the results obtained considering all *Legionella* spp. by culture and PCR for the 46 potting soils analysed

Culture	PCR		
	Positive	Negative	Total
Positive	19 (41.3%)	2 (4.3%)	21 (45.7%)
Negative	22 (47.8%)	3 (6.5%)	25 (54.3%)
Total	41 (89.1%)	5 (10.9%)	46 (100%)

Discussion

This study has demonstrated that *Legionella* spp. are present in commercial potting soils available on the Swiss market. The 46 samples analysed reflect the potting soils readily available in the better-known supermarkets in all parts of the country, and they give a convincing picture of the *Legionella* contamination of potting mixtures sold in Switzerland.

High levels of legionellae were detected in the soil samples. These ranged from 10^3 CFU/g to 10^5 CFU/g (culture method) and from 10^4 GU/g to 10^6 GU/g (qPCR method). The culture and molecular detection methods used showed similar detection limits, with qPCR giving more positive samples. The molecular method allows the detection of very low numbers of legionellae, including non-cultivable strains, and cells inhibited by the antibiotics contained in the selective media against a high background of soil microorganisms. On the other hand, this method does not distinguish between viable and non-viable or dead bacterial cells. All these factors could explain the discrepancy between culture and PCR (22 negative cultures that were positive by PCR).

In this study, isolates belonged to pathogenic species of *Legionella* (*L. pneumophila* 1, *L. pneumophila* 2–15, *L. bozemanii*, *L. micdadei*, *L. longbeachae*, *L. sainthelensi*, *L. feelei*, and *L. birminghamensis*) and to non-pathogenic species (*Legionella gratiana*, *L. jamestowniensis*, *L. nautarum*, *L. quinlivanii*, and *Legionella londiniensis*), as well as to two undefined species that have been designated in the *mip* database as species B-IMVS-86 and strain 81-029 [11]. Species B-IMVS-86 has a homology of 80.3% with *Legionella oakridgensis*, and strain 81-029 a homology of 88.5% with *L. gratiana*. Considering the wide range of species found in the potting mixtures, it could be interesting to test more colonies of the same morphotype.

The predominant species in the soils analysed was *L. bozemanii*, which was isolated from 12 samples (26.1%). Whereas *L. longbeachae* was the most frequently isolated species (58%) in potting soils in Australia, and is responsible for 30% of reported cases of LD [1], only 4.3% of the Swiss potting soils contained this species, a value closer to that reported for Japanese potting soils (8.3%) [12]. So far, no cases of *L. longbeachae* infection have been reported in Switzerland (Swiss Federal Office of Public Health, personal communication). On the other hand, the isolation rates for *L. pneumophila*, responsible for 90% of the reported cases in Europe [11], are higher in Swiss (*L. pneumophila* 1–15, 19.6% of samples) than in Japanese (*L. pneumophila* 2–14, 4.2% of samples) or Australian (*L. pneumophila* 1–14, 13.3% of samples) potting soil. It is important to highlight that 6.5% of the samples contained *L. pneumophila* 1, which is responsible for approximately 70% of LD cases in Europe [11].

Legionella strains were detected (culture isolation and/or PCR detection) from nearly all potting soil samples tested (93.5%).

Two-thirds of the analysed samples contained peat moss. This is in contrast with other studies reporting that legionellae were never isolated from samples containing peat [2,12].

It is unclear how legionellae from potting soils may infect humans [11], but infection probably occurs through inhalation of bio-aerosol formed during gardening activities (direct manipulation of the potting soil, and sprinkling) [13] or after exposure of the soil to meteorological factors such as wind and rain [14]. In a preliminary experiment, *L. bozemanii* could be detected in the air sampled during manipulation of potting soil by exposure of GVPC medium to the air (data not shown). This observation supports the possibility of contamination by inhalation of bio-aerosol produced during these activities.

A limitation of this study is that we did not use the amoebal enrichment method for the analysis of samples. This method is more sensitive than the direct culture method, and is able to detect very low concentrations of legionellae and enhance the cultivability of some *Legionella* spp. [15]. This was demonstrated in a recent study where the amoebal enrichment method detected the presence of 10^2–10^4 CFU/g of legionellae in 91.6% of the potting soils tested [12]. Despite this limitation, the use of qPCR allowed us to obtain comparable sensitivity to that of the amoebal enrichment method: *Legionella* spp. were detected in nearly all potting soils (89.1%).

Further work is underway to include the amoebal enrichment culture method to improve the rate of isolation of *Legionella* spp. from potting soil samples.

In conclusion, potting soils could be considered as an alternative and important source of infection (probably underestimated) not only by *L. longbeachae* but also by other *Legionella* spp. known to cause LD. The Swiss Federal Office of Public Health has currently added potting soils as a potential source of infection to the form used to collect epidemiological data for its mandatory LD notification system. We believe that epidemiological investigation of future LD cases should include collection of environmental samples, in particular potting soils, in addition to water samples.

Acknowledgements

We thank the Federal Office of Public Health for its continued collaboration and support. We also thank A. Demarta (Istituto cantonale di microbiologia, Bellinzona, Switzerland), O. Petrini (Istituto cantonale di microbiologia, Bellinzona, Switzerland), S. Graf (Federal Office of Public Health, Bern, Switzerland) and S. Lai (Health Protection Agency, London, UK) for constructive advice.

Transparency Declaration

The authors declare that they have no conflicts of interest, financial or other.

References

1. Steele TW, Lanser J, Sangster N. Isolation of *Legionella longbeachae* serogroup 1 from potting mixes. *Appl Environ Microbiol* 1990; 56: 49–53.
2. Steele TW, Moore CV, Sangster N. Distribution of *Legionella longbeachae* serogroup 1 and other *Legionellae* in potting soils in Australia. *Appl Environ Microbiol* 1990; 56: 2984–2988.
3. Hughes MS, Steele TW. Occurrence and distribution of *Legionella* species in composted plant materials. *Appl Environ Microbiol* 1994; 60: 2003–2005.
4. Wallis L, Robinson P. Soil as a source of *Legionella pneumophila* serogroup 1 (Lp1). *Aust NZ J Public Health* 2005; 29: 518–520.
5. den Boer JW, Yzerman EP, Jansen R *et al.* Legionnaires' disease and gardening. *Clin Microbiol Infect* 2007; 13: 88–91.
6. Koide M, Saito A, Okazaki M, Umeda B, Benson RF. Isolation of *Legionella longbeachae* serogroup 1 from potting soils in Japan. *Clin Infect Dis* 1999; 29: 943–944.
7. Anonymous. Legionnaires' disease associated with potting soil—California, Oregon, and Washington, May–June 2000. *MMWR* 2000; 49: 777–778.
8. Helbig JH, Bernander S, Castellani Pastoris M *et al.* Pan-European study on culture-proven legionnaires' disease: distribution of *Legionella pneumophila* serogroups and monoclonal subgroups. *Eur J Clin Microbiol Infect Dis* 2002; 21: 710–716.
9. Ratcliff RM, Lancer JA, Manning PA, Heuzenroeder MW. Sequence-based classification scheme for the genus *Legionella* targeting the mip gene. *J Clin Microbiol* 1998; 36: 1560–1567.
10. Ratcliff RM, Slavin MA, Sangster N, Doyle RM, Seymour JF, Lanser JA. *Legionella pneumophila mip* gene sequencing to investigate a cluster of pneumonia cases. *Pathology* 2003; 35: 65–69.
11. Bartram J, Chartier Y, Lee JV, Pond K, Surman-Lee S. *Legionella and the prevention of legionellosis*. Geneva, Switzerland: WHO, 2007.
12. Koide M, Arakaki N, Saito A. Distribution of *Legionella longbeachae* and other *Legionellae* in Japanese potting soils. *J Infect Chemother* 2001; 7: 224–227.
13. O'Connor BA, Carman J, Eckert K, Tucker G, Givney R, Cameron S. Does using potting mix make you sick? Results from a *Legionella longbeachae* case–control study in south Australia. *Epidemiol Infect* 2007; 135: 34–39.
14. Jones AM, Harrison RM. The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. *Sci Total Environ* 2004; 326: 151–180.
15. Koziol-Montewka M, Magrys A, Stojek N *et al.* Monitoring Legionella species in hospital water systems. Link with disease and evaluation of different detection methods. *Ann Agric Environ Med* 2008; 15: 143–147.