BRIEF COMMUNICATIONS AAEM

Ann Agric Environ Med 2002, 9, 261–264

LEGIONELLA IN SPRINKLING WATER AS A POTENTIAL OCCUPATIONAL RISK FACTOR FOR GARDENERS

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Stojek NM, Dutkiewicz J: *Legionella* in sprinkling water as a potential occupational risk factor for gardeners. *Ann Agric Environ Med* 2002, **9**, 261–264.

Abstract: To study a possibility of infection with *Legionella* at gardening by inhaling of water aerosolized at sprinkling of plants, samples of tap water used for sprinkling of plants grown in outdoor gardens and in greenhouses in the Lublin province (eastern Poland) were examined for the presence of *Legionella*, along with the samples of soil, artificial medium and air collected in modern greenhouses. The strains of *Legionella* were isolated from 8 out of 36 samples of water (22.2%) collected from outdoor taps used for sprinkling plants cultivated in outdoor gardens, and from 5 out of 20 samples of water (25.0%) collected from indoor taps used for sprinkling of plants cultivated in traditional greenhouses or foil tunnels. Both in the samples collected from outdoor and indoor taps *Legionella pneumophila* 2–14 was more common than *Legionella* spp. (respectively 13.9% *vs.* 8.3%, and 15.0% *vs.* 10.0%). No legionellae were found in 18 samples of tap water, 14 samples of soil, 14 samples of artificial medium or 6 samples of air collected in modern greenhouses. The results of this preliminary study suggest that water aerosolized at sprinkling of plants represents a potential source of *Legionella* infection among gardeners.

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Key words: *Legionella*, water, gardeners, greenhouses, sprinklers, occupational exposure.

INTRODUCTION

Legionella is a fastidious Gram-negative bacterium developing mostly in water, less often in soil and organic matter [6, 24, 31]. Some species are pathogenic for humans and may cause respiratory disease (legionellosis, Legionnaires' disease), appearing as an atypical pneumonia or influenza-like illness (Pontiac fever), after inhalation of bacteria-laden droplet aerosol or dust [10, 24, 29]. People exposed to infectious aerosol when bathing and performing various occupations are liable to contract legionellosis [6, 12, 20, 24, 31]. The increased risk of exposure to *Legionella* was reported among workers employed at cooling towers [14, 17], oil drilling platforms [8, 21], automotive plants [11], plastics factories [1], ship repair facilities [9], sewage treatment plants [16], and dental facilities [2, 23], as well as among gardeners [7, 22], miners [3], turbine operators [13], construction workers [15], subway personnel [15], railroad conductors [15].

The until recently described cases of legionellosis among professional and amateur gardeners, some with fatal outcome [10, 18, 22, 26], were mostly associated with exposure to potting soil and its components (sawdust, composted leaves) contaminated with *Legionella longbeachae* [7, 10, 19, 25, 27, 28]. The role of water aerosolized at sprinkling of plants as a potential source of infection is less well known. To explore this possibility, a preliminary study was made in which samples of tap water used for sprinkling of plants grown in outdoor gardens and in greenhouses were examined for the presence of *Legionella*, along with the samples of soil, artificial medium and air collected in modern greenhouses.

Received: 18 September 2002
Accepted: 19 November 2002

Table 1. Occurrence of *Legionella* in environmental samples collected on gardening farms in Lublin province.

Source of Samples	Locality	*Legionella pneumophila* 2-14 Positive/examined (Percent)	*Legionella* spp. Positive/examined (Percent)	Total *Legionella* Positive/examined (Percent)
Water: outdoor taps	1	0/4	0/4	0/4
	2	2/9	1/9	3/9
	3	2/12	2/12	4/12
	4	0/3	0/3	0/3
	5	1/4	0/4	1/4
	6	0/2	0/2	0/2
	7	0/2	0/2	0/2
Total		5/36 (13.9%)	3/36 (8.3%)	8/36 (22.2%)
Water: taps in traditional greenhouses	1	2/2	0/2	2/2
	2	1/3	1/3	2/3
	3	0/6	1/6	1/6
	4	0/1	0/1	0/1
	5	0/8	0/8	0/8
Total		3/20 (15.0%)	2/20 (10.0%)	5/20 (25.0%)
Water: taps in modern greenhouses	7	0/6	0/6	0/6
	8	0/12	0/12	0/12
Total		0/18 (0)	0/18 (0)	0/18 (0)
Soil: modern greenhouse	8	0/14 (0)	0/14 (0)	0/14 (0)
Total		0/14 (0)	0/14 (0)	0/14 (0)
Artificial medium: modern greenhouse	8	0/14 (0)	0/14 (0)	0/14 (0)
Total		0/14 (0)	0/14 (0)	0/14 (0)
Air: modern greenhouse	8	0/6 (0)	0/6 (0)	0/6 (0)
Total		0/6 (0)	0/6 (0)	0/6 (0)

MATERIALS AND METHODS

Samples. Altogether, 108 samples were collected from gardening farms located on the territory of 8 villages (1-8) in Lublin province (eastern Poland) and examined for the presence of *Legionella* (Tab. 1). The samples comprised:

- 36 samples of water collected from outdoor taps located on 36 farms, providing water for sprinklers used for watering plants in outdoor gardens (villages 1-7);
- 20 samples of water collected from indoor taps located in 20 small, traditional greenhouses or foil tunnels (in which plants were grown an potting soil), providing water for sprinklers used for watering plants in these greenhouses (villages 1-5);
- 18 samples of water collected from 20 indoor taps located in 2 large, modern greenhouses (in which plants were grown on soil or artificial medium), providing water for sprinklers used for watering plants grown on soil, or for other purposes (villages 7, 8);
- 14 samples of soil collected in a modern greenhouse (village 8);
- 14 samples of artificial medium collected in a modern greenhouse (village 8);
- 6 samples of air collected in a modern greenhouse (village 8).

Processing of samples. Water samples of 300 ml volume were filtered through cellulose filters (pores 0.45 μm, Millipore, USA). Filters were washed for 10 min in acid buffer (pH 2.2), then rinsed in Ringer solution (Merck, Germany) and placed on isolation agar medium.

Samples of potting soil were suspended in Ringer solution with glass beads (proportion 10 g/50 ml), manually shaken for 10 min, and centrifuged for 20 min at 5,000 rpm. Supernatant (2 ml portions) was heated at 50ºC for 30 min and spread on the surface of the isolation agar medium in inoculation doses of 0.5 ml per plate. Artificial medium samples were processed in the same manner except that they were crushed with sterile tweezers during collection.

The air samples were taken on glass fiber filters (Type A/E, Gelman Sciences Inc., USA) with the personal sampler

AP-2 (TWOMET, Zgierz, Poland), in the volume of 60 l/filter. Samples were extracted in 2 ml of saline (0.85% NaCl supplemented with 0.05% Tween 80) for 2 hrs. Extract was heated at 50ºC for 30 min and spread on the surface of the isolation agar medium in inoculation doses of 0.5 ml per plate, as above.

Isolation and identification of *Legionella* strains. The buffered charcoal yeast extract (BCYE) agar medium [6, 31] supplemented with the Growth Supplement SR 110 A and the Selective GVPC Supplement SR 152 E (Oxoid, England) was used for isolation of *Legionella*. Inoculated agar plates were incubated for 7 days at 37ºC with daily check of growth. Colonies of Gram-negative bacteria grown after 4–7 days were isolated and examined for ability to grow on media with and without cysteine. Strains unable to grow on media without cysteine were considered as suspected *Legionella* strains. The isolates were determined to the species and serogroup level with the use of the Legionella Latex Test Kit (Oxoid, England) which enables, on the basis of microagglutination with latex particles sensitised with specific rabbit antibodies, a separate identification of *Legionella pneumophila* serogroup 1, *Legionella pneumophila* serogroups 2-14, and *Legionella* spp. (a complex group including: *L. longbeache* serogroups 1 and 2, *L. bozemanii* serogroups 1 and 2, *L. dumoffii, L. gormanii, L. jordanis, L. micdadei* and *L. anisa*). Only isolates giving positive reaction in the latex test were considered as strains of *Legionella*.

RESULTS

The results of the study are presented in Table 1. Incidence of *Legionella* in the samples of water from outdoor taps used for sprinkling of plants cultivated in outdoor gardens and from indoor taps used for sprinkling of plants cultivated in traditional greenhouses was similar, being respectively 22.2% and 25.0%. Both in the water samples from outdoor and indoor taps *Legionella pneumophila* 2-14 was more common than *Legionella* spp. (respectively 13.9% *vs.* 8.3%, and 15.0% *vs.* 10.0%). Altogether, legionellae were found in water from outdoor taps in 3 out of 7 examined villages, whereas in water from indoor taps in greenhouses – in 3 out 5 of examined villages. No legionellae were found in the samples of tap water, soil, artificial medium and air collected in modern greenhouses.

DISCUSSION

Inhalation of potting soil contaminated with legionellae is considered as the main route of legionellosis transmission to gardeners, while the role of exposure to aerosolized water is less well known in this respect.

Legionella longbeachae and other *Legionella* species (*L. bozemanii, L. micdadei, L. pneumophila, L. anisa*) were commonly isolated from the samples of potting soil and its constituents in Australia [7, 25, 27, 28], Japan [19] and the USA [10]. Steele *et al.* [27] isolated *L. longbeachae* serogroup 1 from Australian samples of soil and potting mixes, but not from water samples. No *Legionella* strains were isolated from the samples of potting soil collected in Europe [10, 28], and from the samples of peat moss which is commonly used in Europe for enrichment of potting soils [19].

This study indicates that besides potting soils, tap water aerosolized by garden sprinklers may be also a potential source of *Legionella* infection in gardeners. The risk seems to be greater for gardeners working in outdoor gardens and traditional greenhouses, but much lower for those working in the modern greenhouses where artificial media receiving water through a capillary system are used for plant cultivation. Exposure to a similar type of water aerosol generated by display whirlpool spas has been identified as a cause of legionellosis outbreaks [4, 5]. Boshuizen *et al.* [5] described subclinical legionellosis in floral exhibitors exposed to a whirlpool spa. This was manifested by rise in anti-*Legionella* antibody titer that was greater in people working closer to the source of droplet aerosol. The authors express the opinion that only 0.1–5% of individuals exposed to *Legionella* develop clinical symptoms [5].

A limitation of this study is that the microbiological study of water samples was not accompanied by serological examination of gardeners. In an earlier serological survey [30] it was evidenced that the prevalence of anti-*Legionella* antibodies among the inhabitants of village # 3 (both gardeners and non-gardeners) was high (34.3%), significantly greater compared to city dwellers ($p < 0.001$). In the present study, *Legionella* has been isolated from tap water samples collected in this village, both from outdoor taps (in 33.3% of total samples) and indoor taps in greenhouses (in 16.7% of total samples). It is intended to continue the study with the use of a more sensitive PCR method for *Legionella* detection and the serological testing of exposed gardeners *versus* non-exposed rural inhabitants.

In conclusion, results of this preliminary study suggest that water aerosolized at sprinkling of plants represents a potential source of *Legionella* infection among gardeners.

Acknowledgements

The authors wish to thank Professor Dr habil. Hanna Stypułkowska-Misiurewicz, Dr Bożena Krogulska and Dr Renata Matuszewska from the National Institute of Hygiene in Warsaw for their most valuable help and advice in the initiation and performance of this study.

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