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Research note

Green waste compost as potential reservoirs of *Legionella* in the Netherlands

A. Huss*, L.A.N. Derks, D.J.J. Heederik, I.M. Wouters

Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

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ABSTRACT

Objectives: *Legionella* is a bacterial species able to cause influenza-like illness (Pontiac fever) or severe pneumonia (Legionnaires disease, LD). We assessed *Legionella* presence and concentration in composting facilities in The Netherlands.
Methods: A total of 142 samples from 23 green waste composting facilities were screened for *Legionella* DNA using qPCR.
Results: Of 142 samples, *Legionella* spp. DNA was detected in 97 (68%), and the subspecies *L. pneumophila* and *L. longbeachae* in 33 (23%) and one (0.7%) samples, respectively. *Legionella* was observed in samples from all composting facilities. The concentration of *Legionella* spp. DNA ranged from 10^3 to 10^5 genomic units (GU)/gram. Compost temperature was negatively correlated with the presence (odds ratio 0.67, 95% CI 0.50–0.92 per 10 degrees increase) and concentration (geometric mean ratio 0.90, 95% CI 0.83–0.97 per 10 degrees) of *Legionella* spp. Average humidity in the week prior to sampling was negatively correlated with the *L. pneumophila* concentration (geometric mean ratio 0.73, 95% CI 0.56–0.96 per increase in 10% of humidity).
Discussion: This study suggests that composting facilities can be regarded as reservoirs of *Legionella* in The Netherlands, but additional studies should target if such facilities represent a human health risk.
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Introduction

Legionellae are Gram-negative bacterial species able to cause influenza-like illness (Pontiac fever) or severe pneumonia (Legionnaire's disease, LD) in humans [1,2]. Known infection sources include aerosols from contaminated aquatic systems such as cooling towers, air conditioners, etc. However, in most cases it remains unclear which source was responsible for the infection [2]. Since 2012, there has been a clear increase in the incidence of LD in The Netherlands, highlighting the relevance of further research into possible sources of infection. Over 60 species of *Legionella* have been identified to date [2]. By far the majority of the European LD cases with a known cause are due to *L. pneumophila*, whereas in Australia and New Zealand *L. longbeachae* has been observed to play a major role in LD incidence as well [3]. Green waste compost and potting soils can contain Legionellae [4]. Multiple clinical cases of potting soil- or compost-associated infection have been shown to be caused by *L. longbeachae* [3,5]. With its adapted genome, *L. longbeachae* is better equipped for survival in soil-like environments [5]. In The Netherlands, it is unclear if composting facilities may represent potential reservoirs of *Legionella*. We aimed at assessing if green waste compost on Dutch composting facilities contains *Legionella*, to estimate the fraction composed of *L. pneumophila* and *L. longbeachae*, and to identify predictive factors for the presence of *Legionella*.

Methods

We contacted green waste composting facilities in The Netherlands ($n = 53$) from a list of the BVOR (Branche Vereniging Organische Reststoffen) supplemented with facilities found on the Internet; 23 facilities (43%) agreed to participate in our study. Information about the composting process was collected when visiting the facilities (Table S1). Weather data from the closest

* Corresponding author. Anke Huss, Institute for Risk Assessment Sciences, Utrecht University, Yalelaan 2, 3584 CM Utrecht, the Netherlands.
E-mail address: a.huss@uu.nl (A. Huss).

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weather station was downloaded from the Royal Netherlands Meteorological Institute (KNMI; Table S2).

At each facility, ten scoop samples were collected separately at the surface and at 30 cm depth from (a) fresh green waste after grinding, (b) material halfway in the composting process and (c) the end product, and subsequently pooled and mixed, resulting in six samples per facility. At two facilities, additional samples were collected from the end product as storage location and sieving size varied. A total of 142 pooled samples were collected between February and April 2019.

As inhibition of *Legionella* during culture has been described [1,7,8], *Legionella* was determined by qPCR. DNA was extracted from samples of 0.25 g using the QiaGen DNeasy PowerSoil kit. Fresh waste samples were chopped in a Moulinex grinder prior to DNA extraction. qPCR was performed to detect generic *Legionella* spp. (ssrA gene) and *L. pneumophila* (mip gene) and *L. longbeachae* (gyrB gene) as previously described by Nasir et al., 2018 [7], applying the qPCRs described by Collins et al. [6] and the genesig® *L. longbeachae* standard kit.

We evaluated if weather (air temperature, precipitation or humidity in the week prior to sampling; all continuous variables) or sample (temperature, pH; both continuous variables) or production-related factors (fresh, halfway or end product; turning frequency: 2 to >10 times); turning method (shovel, excavator, compost turner, combination), amount of annual waste input per year (1700–100 000 t/a); composting method (heap, windrows, combination) (all production-related factors were categorical variables) represented predictors of log-transformed concentration using simple linear regression, or presence versus absence using logistic regression of *Legionella* spp. as well as *L. pneumophila*. In these regression models, we evaluated all variables listed above, one at a time. Analyses were performed in Stata 14, Stata Corp, College Station, TX, USA.

Results

Facilities composted through compost heaps ($n = 14$), windrows ($n = 7$) or both ($n = 2$). *Legionella* spp. DNA were detected in 68% of samples, and DNA of the species *L. pneumophila* and *L. longbeachae* in 23% and 0.7%, respectively. At all 23 participating composting facilities, *Legionella* DNA was found in at least one, but in most cases in three or more samples. *Legionella pneumophila* was found at 19 facilities, while *L. longbeachae* was only found at one. The number and percentages of positive samples are shown in Table 1.

Concentration of *Legionella* spp. in the positive samples ranged from 7.02×10^3 to 7.51×10^5 genomic units (GU)/gram (geometric mean: 7.78×10^4 GU/gram). Concentration of *L. pneumophila* in the 33 positive samples ranged from 2.14×10^3 to 2.85×10^4 GU/gram (geometric mean: 7.05×10^3 GU/gram). Fresh green waste at 30 cm depth had fewer *L. pneumophila*-positive samples than the other sampling sites. The concentration of *L. longbeachae* DNA in the only positive sample was 1.09×10^4 GU/gram.

Logistic and linear regression indicated only sample temperature to be negatively associated with the presence (odds ratio 0.67, 95% CI 0.50–0.92 per increase in 10 degrees) and concentration (geometric mean ratio 0.90, 95% CI 0.83–0.97 per increase in 10 degrees) of *Legionella* spp. (Fig. 1). For *L. pneumophila,* average air humidity in the week prior to sampling was negatively associated with the concentration of *L. pneumophila* in positive samples (geometric mean ratio 0.73, 95% CI 0.56–0.96 per increase in 10% of humidity). Odds were higher for detecting *L. pneumophila* in samples that came from halfway or the end of the production process as compared to fresh samples (odds ratios 5.6, 95% CI 1.7–18.4 and 3.7, 95% CI 1.1–12.3, respectively).

Discussion

We observed Legionella DNA at all composting facilities included in our study. Sixty-eight per cent of individual samples contained *Legionella* spp. and 23% *L. pneumophila. Legionella longbeachae* was observed in one sample out of 142 (0.7%). Sample temperature was associated with *Legionella* spp., with increasing temperature translating to fewer positive samples as well as to lower bacteria concentrations in positive samples.

Limitations pertain to performing qPCR, which does not provide information on viability, yet it is the best option for compost samples: culturing underestimates presence of *Legionella* due to inhibition or overgrowth by other microorganisms [8], especially for compost samples which contain a vast amount of thermotolerant microorganisms [1,7]. Besides, hazardous *Legionella* in a viable but non-culturable state, will be detected by qPCR [8].

Our findings are in line with those from two Swiss assessments that recovered *Legionella* isolates at 69% and 75% of the included compost facilities, respectively through culture [4,9]. Casati et al. reported fresh green waste to be free of *Legionella* spp. and suggested that contamination could be brought about by wind and rain [9]. While we found *L. pneumophila* less frequently in fresh green waste, the percentage of samples in which we detected *Legionella* spp. did not differ by age of the material. This may be caused by outside storage of fresh green waste before processing or the difference in detection method. Regarding associations with compost temperatures, one previous study observed that compost samples with higher temperatures were less likely to be *Legionella* spp. positive [4], similar to our findings. Water-cultured *L. pneumophila* at temperatures between 25 and 45 degrees was reported to grow strongest at 35 degrees [10], but this was not a trend we could clearly observe in our compost samples.

In conclusion, green waste compost appears to represent a reservoir of *Legionella*. Given recent increases in reported LD cases, it is of relevance to also evaluate non-aquatic systems as possible sources of infection. Bioaerosols can be released during green

Table 1
Overview of the numbers and percentages of samples positive for Legionella spp., L. pneumophila and L. longbeachae, categorized by sampling location

Sampling location		Number of samples	Number and percentage of positive samples		
			Legionella spp.	L. pneumophila	L. longbeachae
Fresh	Surface	23	18 (78)	4 (17)	0
	30 cm	23	17 (74)	1 (4)	0
Halfway	Surface	23	18 (78)	10 (43)	1 (4)
	30 cm	23	12 (52)	6 (26)	0
End	Surface	24	15 (63)	5 (21)	0
	30 cm	26	17 (65)	7 (27)	0
Total		142	97 (68)	33 (23)	1 (0.7)

Data are presented as *n* (%).

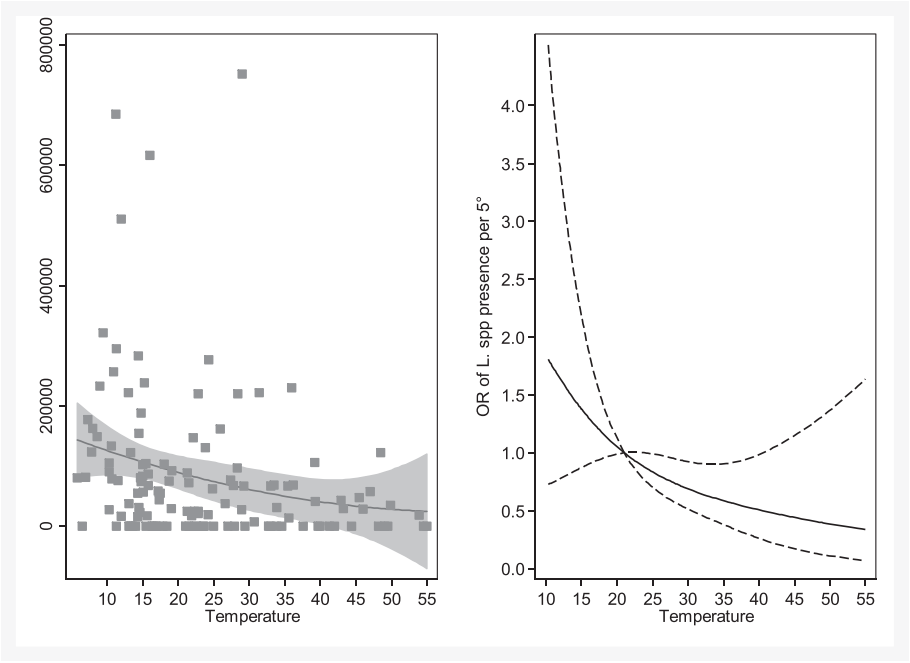

Fig. 1. Legionella *spp.* concentration and risk of presence by sample temperatures (°C). Left: *Legionella* spp. concentration vs. sample temperature, the dots represent concentration in GU/gram. Right: *Legionella* spp. restricted cubic splines odds ratio of presence per 5°sample temperature; dashed lines represent upper and lower confidence intervals, 21° (the median of all samples' temperatures) used as referent.

composting agitation processes [11–13]. The next steps should consider seasonal differences, and if occupational contact with compost, residential proximity to composting facilities or handling of the end product (i.e. potting soil), translates to human *Legionella* exposure and possible health risk.

Transparency declaration

The authors declare that they have no conflicts of interest, financial or other. This study was funded with intramural funds of the University of Utrecht.

Authors contributions

A.H. is lead and corresponding author. Writing and original draft: L.A.N.D. and A.H. Interpretation of data, Review and Editing: All authors. Conceptualization: I.M.W. and A.H. Investigation: L.A.N.D. Methodology: I.M.W. and L.A.N.D. Formal analysis: L.AN.D. and A.H.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.05.018.

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