Environmental Science & Technology

Critical Review

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Confirmed and Potential Sources of *Legionella* Reviewed

Eri van Heijnsbergen,*,† Johanna A. C. Schalk,† Sjoerd M. Euser,‡ Petra S. Brandsema,† Jeroen W. den Boer,‡ and Ana Maria de Roda Husman†,§

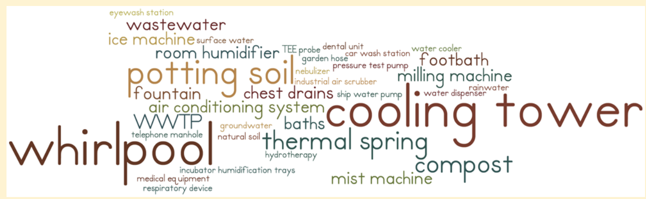
†National Institute for Public Health and the Environment, A. van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands

‡Regional Public Health Laboratory Kennemerland, Haarlem, Boerhaavelaan 26, 2035 RC Haarlem, The Netherlands

§Institute for Risk Assessment Sciences, Utrecht University, Yalelaan 2, 3584 CM Utrecht, The Netherlands

Supporting Information

ABSTRACT: *Legionella* bacteria are ubiquitous in natural matrices and man-made systems. However, it is not always clear if these reservoirs can act as source of infection resulting in cases of Legionnaires' disease. This review provides an overview of reservoirs of *Legionella* reported in the literature, other than drinking water distribution systems. Levels of evidence were developed to discriminate between potential and confirmed sources of *Legionella*. A total of 17 systems and matrices could be classified as confirmed sources of *Legionella*. Many other man-made systems or natural matrices were not classified as a confirmed source, since either no patients were linked to these reservoirs or the supporting evidence was weak. However, these systems or matrices could play an important role in the transmission of infectious *Legionella* bacteria; they might not yet be considered in source investigations, resulting in an underestimation of their importance. To optimize source investigations it is important to have knowledge about all the (potential) sources of *Legionella*. Further research is needed to unravel what the contribution is of each confirmed source, and possibly also potential sources, to the LD disease burden.



■ INTRODUCTION

Legionella are Gram-negative bacteria that cause Legionnaires' disease (LD) and Pontiac fever in humans.[1] *Legionella* bacteria are ubiquitous in the natural environment in both soil and water. *Legionella* infections are regularly traced to contaminated man-made water systems, such as water distribution systems,[2,3] cooling towers[4] and whirlpools.[5] These systems often exhibit favorable growth conditions for *Legionella*. Transmission to humans occurs via contaminated water aerosolization.[1] Potting soil is also an infection source,[6] but the mode of transmission of *Legionella* originating from this source remains unclear. *Legionella pneumophila*, predominantly *L. pneumophila* serogroup 1 (SG1), constitutes over 90% of the clinical isolates in Europe[7,8] and the U.S.[9] In Australia, New Zealand, and Thailand, *L. longbeachae* is an important cause of LD.[10−12]

Epidemiological studies are conducted to identify the infection source of sporadic LD cases or outbreaks, and microbiological methods are employed to isolate *Legionella* from suspected sources for confirmation. Molecular tracing is used to establish a link between clinical *Legionella* isolates and environmental isolates. However, the infection source remains unknown for most sporadic LD cases.[13] Moreover, studies in The Netherlands, England and Wales showed that only a few sequence types of *L. pneumophila* cause the majority of sporadic *Legionella* infections, whereas these particular sequence types are only rarely detected in suspected sources.[14,15] The failure of standard culture methods to detect virulent strains in environmental samples could explain this discrepancy. *Legionella* bacteria are typically cultured on buffered charcoal yeast extract (BCYE, with or without antibiotics) medium plates for detection.[16] False negative results can occur because these plates are easily overgrown by other bacteria in the sample or because *Legionella* bacteria in a viable-but-not-culturable (VBNC) state are not detected. Furthermore, virulent *Legionella* strains might only be present in low concentrations in the environment compared to other less virulent strains, thus masking their detection. Another explanation is that important *L. pneumophila* sources are not considered during source investigations.[17]

To optimize LD source investigations it is important to have knowledge about all the reservoirs of *Legionella*, and whether exposure to these reservoirs can lead to infection. In the present study, a literature review was systematically conducted to obtain an overview of all reservoirs and sources of *Legionella*. The matrices and systems that are described in literature were classified according to the strength of evidence that implicates that the matrix or system could be a source of *Legionella* infection. For this purpose a level of evidence (LOE) approach was used. LOE approaches are used in evidence-based medicine[18] and other research areas such as waterborne infectious disease surveillance.[19] These approaches did not fit our specific research question and therefore LOEs were developed and assigned to every selected publication. Based

Received: January 12, 2015
Revised: March 9, 2015
Accepted: March 16, 2015
Published: March 16, 2015

on the assigned LOEs, systems and matrices were classified as potential source or confirmed source. A source was classified as confirmed if at least in one study sufficient evidence was provided that the described source was the cause of infection. Drinking water sources, such as showers and taps, are often targeted in outbreak investigations, and in many countries, legislation is already in place for preventive measures. Therefore, those sources are outside the scope of this review.

■ MATERIALS AND METHODS

Literature Search. A literature search was conducted using the MEDLINE database (publisher: USA National Library of Medicine), searched by OvidSP (Wolters Kluwer Health). The search was performed on June 25, 2013. The following terms were used to select publications about *Legionella*, LD or Pontiac fever: in the title and abstract: 'legionell*' OR 'legionnair*' OR 'Pontiac fever'; and in Medical Subject Headings (MeSH): 'exp Legionellosis/' OR 'exp *Legionella*/'. These terms were combined with an extensive list of additional terms to identify publications that reported sources and reservoirs of *Legionella*. No restrictions for the publication date were imposed. PubMed (publisher: USA National Library of Medicine) was searched in addition to the MEDLINE database because the former also covers publications that are electronically published ahead of print. The same MeSH terms were applied, but only the publication titles, not the abstracts, were searched for the terms 'legionell*' OR 'legionnair*' OR 'Pontiac fever'. These terms were not combined with additional search terms. The publication date criterion was between June 1, 2012 and June 25, 2013.

Study Inclusion Criteria. A study was included if it was written in English, reported on primary research results and fulfilled at least one of the following criteria: 1. the study described the detection of *Legionella* spp. in environmental sources in surveillance or prevalence studies or in source investigations; 2. the study described risk factors that could be related to exposure to *Legionella* sources; 3. epidemiological data on LD or Pontiac fever cases were used to identify a possible infection source. Publications describing showers or taps as sources or reservoirs of *Legionella* were excluded (as discussed above). However, the indirect use of drinking water was included, for example, the use of tap water for cleaning purposes, rinsing medical equipment or dental units. The use of tap water in baths was also included because baths involve the use of water for a prolonged period at a certain temperature, which might promote the *Legionella* growth.

Selection Process. The selection process was conducted in two steps. First, the title and abstract of all publications were assessed independently by three researchers (SE, EH, and JS). Publications that were not relevant for the research objective were not selected for full-text assessment. In the second selection step, the full-text versions of the selected publications were assessed for eligibility on the basis of the selection criteria by one reviewer (EH). When multiple studies on the same outbreak were present, only the report providing the highest LOE was included, unless the other studies provided additional information resulting in a higher LOE.

Levels of Evidence. LOEs were developed to discriminate between potential sources and confirmed sources of *Legionella*. Table 1 shows the different LOEs, from I, representing the highest LOE, to VI, representing the lowest LOE. For the highest LOE, cases must be epidemiologically linked to a suspected source and a match, either molecularly (LOE Ia) or by monoclonal antibody typing (LOE Ib), must be determined between the clinical and environmental isolates. Furthermore, there should be additional evidence demonstrating that these sources caused the infection: evidence that excludes other possible sources, evidence on the spread of *Legionella* from the suspected source, or evidence on the exposure of cases to the suspected source. The exclusion of other sources can be achieved by the environmental investigation of other sources, if there is only one common source in an outbreak situation, or if new cases ceased to occur after elimination of the suspected source. Evidence on the spread from the suspect source involves the isolation of *Legionella* from air samples. Evidence of exposure is achieved by case-control studies comparing the seroprevalence of *Legionella* or by case-control studies that imply exposure to the suspected source as a risk factor for contracting LD (in contrast to other considered sources). For LOE II, a match must be identified between clinical and environmental isolates, but additional evidence is not provided. LOE III was assigned when cases were epidemiologically linked to a suspected source, *Legionella* was isolated from this source, and additional evidence was provided, but environmental and clinical strains were not further typed or clinical strains were not available for comparison. LOE IV could be assigned in three situations: (1) cases were epidemiologically linked to a suspected source, and *Legionella* was isolated from this source; (2) cases were epidemiologically linked to a suspected source, no environmental isolates were obtained, but other possible sources were excluded or there was evidence of spread from or exposure to the source; and (3) no cases were linked to a suspected source, but environmental strains were isolated, and additional evidence was provided. When no LD cases were involved, additional evidence could include the isolation of *Legionella* from air samples or case-control studies on the seroprevalence of *Legionella*. LOE V was assigned when *Legionella* was isolated from a reservoir or potential source, or exposure was assessed, or risk factors for contracting LD were determined. LOE VI was assigned to studies in which environmental *Legionella* was not isolated but was detected

Table 1. Levels of Evidence for Sources of *Legionella* Bacteria

level of evidence[a]	cases[b]	environmental *Legionella*	evidence for spread/ evidence for exposure/ other sources excluded	match by molecular method[c,d]	match by monoclonal antibody typing[c]
Ia	×	×[e]	×	×	
Ib	×	×[e]	×		×
IIa	×	×[e]		×	
IIb	×	×[e]			×
III	×	×[e]	×		
IV	×	×[e]			
	×		×		
		×[e]	×		
V		×[e]			
			×		
VI		×[f]			

[a]From high to low. [b]One or more patients are epidemiologically linked to a source. [c]Match is made between environmental and clinical isolate. [d]One of the following molecular methods: PFGE, AFLP, SBT. [e]*Legionella* isolated from reservoirs or (potential) sources. [f]*Legionella* detected in reservoirs or (potential) sources by molecular methods or antibody staining methods only.

DOI: 10.1021/acs.est.5b00142
Environ. Sci. Technol. 2015, 49, 4797−4815

by molecular or antibody staining methods. A study may report the detection of *Legionella* in more than one type of system or reservoir; therefore, one study can be assigned multiple LOEs.

For the described sources and reservoirs of *Legionella*, the LOEs were assessed based on the selected literature. Subsequently, the sources of *Legionella* were subdivided into confirmed sources (at least one publication with LOE Ia or Ib) or potential sources (LOE II or lower).

■ RESULTS

A total of 2189 publications were identified by searching the MEDLINE/PubMed databases, and 1653 were excluded after screening the titles and abstracts. From the remaining publications, 138 were discarded after assessing the full-text, and 398 met the inclusion criteria. Supporting Information Figure S1 shows all of the reservoirs or (potential) sources of *Legionella* that were reported by more than two of the selected publications and the studies that described LD risk factors that could be related to exposure to sources of *Legionella*. Some (potential) sources were only reported in one or two studies ("other"). Table 2 shows the assigned LOEs per potential or confirmed source. In Table 3, all of the references per (potential) source are listed for studies that concerned outbreak investigations or case reports, with an assigned LOE of IV or higher. Cooling towers and whirlpools are not presented in Tables 2 or 3 because not all of the studies were assessed due to the large amount of studies reporting on these sources. Several studies provided the highest LOE for cooling towers and whirlpools, and a selection of these studies is described below. For wastewater, thermal springs, and surface water, all publications were assessed and are presented in Table 2, but only a portion of the publications are described in the text and incorporated in the reference list because of the large amount of studies (see also Supporting Information Tables S4, S5, and S6).

Table 2. Level of Evidence Per Source[a]

	level of evidence						
(potential) *Legionella* source	Ia	Ib	IIa[b]	III	IV	V	VI
potting soil/compost	4			2	3	9	1
baths	3				2	1	
fountains	3			3	1	6	
wastewater/WWTPs	2			3	1	6	6
room humidifiers	2			2	3	1	
ice/ice machines	1	1	1	1		2	
mist machines	1	1				3	
air conditioning systems		2		2		6	2
natural water: thermal springs	1		5	1	3	27	4
natural soil	1				2	9	2
cooling liquid for machinery		1		2			
milling machine	1						
ship water pump	1						
foot bath	1						
underwater chest drain		1					
medical equipment: respiratory devices				3	2	2	
medical equipment: other				2	1	3	
water used for cleaning				1	1	1	
sullage tanks collecting bilge				1			
dental units					2	8	2
roof-harvested rainwater					1	2	3
construction and excavation					1	2	
steam turbine condenser cleaning					1		
inoperative bedpan flusher					1		
natural water: surface water						22	14
natural water: groundwater						7	2
rainwater on the road						3	
steam towel warmer						1	
industrial air scrubber						1	
garden hose						1	1

[a]The numbers in the table reflect the number of studies that were categorized as a certain LOE. Several studies provided the highest level of evidence for cooling towers and whirlpools, however these sources are not presented here because not all studies were assessed due to the great amount of studies reporting on these sources. [b]LOE IIb was never assigned.

■ CONFIRMED *LEGIONELLA* SOURCES

Cooling Towers. Cooling towers were described as a potential or confirmed source in 153 studies. Several of these studies provided the highest LOE (Ia) for cooling towers as an infection source.[20−23] Many outbreaks of LD caused by the transmission of *Legionella* from contaminated cooling towers have been reported; hundreds of individuals have become ill at times.[20,22,24−28] Cooling towers may also be responsible for some of the sporadic community-acquired LD cases. In three studies, an association was observed between the incidence of sporadic community-acquired LD and the proximity of place of residence to a cooling tower.[29−31]

Whirlpools. A total of 36 articles were selected that described whirlpools as a potential or confirmed source. Several studies provided the highest LOE (Ia) in source investigations concerning whirlpools.[32−35] The use of private whirlpools[36,37] and public whirlpools[35,38,39] were linked to patients with LD or Pontiac fever. Several outbreaks of LD were reported in which a whirlpool on display was considered the infection source,[32,34,40,41] and some outbreaks linked to whirlpools occurred on cruise ships.[33,42]

Potting Soil and Compost. In 18 studies, potting soil and/or compost were investigated. LD or Pontiac fever cases were related to exposure to potting soil in nine studies, and nine studies investigated the prevalence of *Legionella* in potting soil or compost. Koide et al.[43] investigated a case related to potting soil and also performed a prevalence study. One study identified risk factors associated with *L. longbeachae* infection.[44]

The infectious agent in all described cases was *L. longbeachae*. The modes of possible exposure primarily included the use of potting soil at home or in the working place. Cramp et al.[45] described one outbreak of Pontiac fever in nine workers of a horticultural warehouse involved in the potting of plants that had the highest LOE (Ia). *L. longbeachae* SG2 isolates from potting soils were indistinguishable by pulsed-field gel electrophoresis (PFGE) from isolates obtained from cases. The water supply of the warehouse was eliminated as a potential source. Three studies reported on clusters or individual cases in which amplified fragment length polymorphism (AFLP) profiles of potting soil isolates and patient isolates were found to yield indistinguishable patterns.[46−48] As other possible sources were excluded, these studies were categorized at the highest LOE (Ia). In eight studies reporting on potting soil as a possible source, no genotypic match was determined, or a source investigation was not conducted.[43,49−55] The results from a case-control study by O'Connor et al.[44] provided some insight into the possible transmission mode of *L. longbeachae*. The predictors of illness in a multivariate analysis included eating or

DOI: 10.1021/acs.est.5b00142
Environ. Sci. Technol. 2015, 49, 4797−4815

Table 3. Levels of Evidence (LOEs) Per Reference Per (Potential) Source[a]

(potential) *Legionella* source	reference	LOE	LD/Pf[b]	number of cases	infective agent
potting soil/compost	Cramp[45]	Ia	Pf	9	*L. longbeachae* SG2
	Den Boer[46]	Ia	LD	1	*L. longbeachae*
	Lindsay[47]	Ia	LD	4[c]	*L. longbeachae* SG1
	Pravinkumar[48]	Ia	LD	3[c]	*L. longbeachae* SG1
	Steele[55]	III	LD	4[c]	*L. longbeachae* SG1
	Speers[49]	III	LD	1	*L. longbeachae*
	CDC[51]	IV	LD	3[c]	*L. longbeachae*
	Dhillon[50]	IV	LD	1	*L. longbeachae*[d]
	Koide[43]	IV	LD	1	*L. longbeachae* SG1
baths	Kura[64]	Ia	LD	3	*L. pneumophila* SG5
	Mineshita[65]	Ia	LD	1	*L. pneumophila* SG5
	Torii[66]	Ia	LD	1	*L. pneumophila* SG10
	Franzin[67]	IV	LD	1	*L. pneumophila*[d]
	Nagai[68]	IV	LD	1	*L. pneumophila*[d]
fountains	Hlady[70]	Ia	LD	5	*L. pneumophila* SG1
	Palmore[72]	Ia	LD	2	*L. pneumophila* SG1
	O'Loughlin[71]	Ia	LD	18	*L. pneumophila* SG1
	Fenstersheib[73]	III	Pf	34	*L. anisa*[d]
	Jones[74]	III	Pf	117	*L. anisa*[d]
	Haupt[76]	III	LD	8	*L. pneumophila*[d]
	Correia[75]	IV	LD	11	*L. pneumophila*[d]
wastewater/WWTPs	Borgen[83]	Ia	LD	5	*L. pneumophila* SG1
	Nygard[84]		LD	56	*L. pneumophila* SG1
	Blatny[87]				
	Blatny[86]				
	Olsen[85]				
	Nguyen[88]	Ia	LD	86	*L. pneumophila* SG1
	Gregersen[89]	III	Pf	5	*L. pneumophila*[d]
	Castor[90]	III	Pf	15	*L. pneumophila*[d]
	Kusnetsov[91]	III	LD	2[c]	*L. pneumophila*[d]
room humidifiers	Yiallouros[107]	Ia	LD	9	*L. pneumophila* SG3
	Moran-Gilad[108]	Ia	LD	1	*L. pneumophila* SG1
	Arnow[104]	III	LD	5	*L. pneumophila*
	Joly[105]	III	LD	5	*L. dumoffii*
	Kaan[106]	IV	LD	1	*L. pneumophila*
ice/ice machines	Bencini[113]	Ia	LD	1	*L. pneumophila* SG1
	Bangsborg[112]	Ib	LD	2	*L. pneumophila* SG1
	Schuetz[115]	II	LD	1	*L. pneumophila* SG8
	Graman[114]	III	LD	1	*L. pneumophila* SG6
mist machines	Barrabeig[119]	Ia	LD	12	*L. pneumophila* SG1
	Mahoney[118]	Ib	LD	33	*L. pneumophila* SG1
air conditioning systems	Breiman[122]	Ib	LD	6	*L. pneumophila* SG1
	O'Mahony[126]	Ib	LD	68	*L. pneumophila* SG1
	Cordes[124]	III	LD	8	*L. pneumophila*[d]
	Kaufmann[125]	III	Pf	144	*L. pneumophila*[d]
natural water: thermal springs	Miyamoto[139]	Ia	LD	1	*L. pneumophila* SG3
	Ito[136]	II	LD	1	*L. pneumophila* SG6
	Kurosawa[137]	II	LD	1	*L. pneumophila* SG1
	Matsui[138]	II	LD	1	*L. rubrilucens*
	Nozue[141]	II	LD	1	*L. pneumophila* SG3
	Tominaga[142]	II	LD	1	*L. pneumophila* SG1
	Gaia[135]	II	LD	1	*L. pneumophila* SG1
	Bornstein[134]	III	LD	5	*L. pneumophila*[d]

DOI: 10.1021/acs.est.5b00142
Environ. Sci. Technol. 2015, 49, 4797−4815

Table 3. continued

(potential) *Legionella* source	reference	LOE	LD/Pf[b]	number of cases	infective agent
	Molmeret[140]	IV	LD	2[c]	*L. pneumophila* SG1
natural soil	Wallis[149]	Ia	LD	1	*L. pneumophila* SG1
	Parry[150]	IV	LD	5	*L. bozemanii*
	Haley[151]	IV	LD	49	LD bacterium
cooling liquid for machinery	Allen[160]	Ib	LD	1	*L. pneumophila* SG1
	Herwaldt[161]	III	Pf	317	*L. feeleii*[d]
	O'Keefe[162]	III	LD, Pf	2	*L. pneumophila*[d]
milling machine	Coscolla[163]	Ia	LD	11	*L. pneumophila* SG1
ship water pump	Cayla[164]	Ia	LD	2	*L. pneumophila* SG1
footh bath	Den Boer[165]	Ia	LD	3	*L. pneumophila* SG1
under water chest drain	Moiraghi[166]	Ib	LD	12	*L. pneumophila* SG1
respiratory devices	Arnow[104]	III	LD	5	*L. pneumophila*
	Joly[105]	III	LD	5	*L. dumoffii*
	Moiraghi[166]	III	LD	12	*L. pneumophila* SG1
	Aubert[167]	IV	LD	1	*L. pneumophila* SG1, SG8
	Pilon[168]	IV	LD	1	*L. pneumophila* SG1
TEE probes	Levy[171]	III	LD	3	*L. pneumophila* SG1
hydrotherapy system	Leoni[170]	III	LD	1	*L. pneumophila*[d]
medication nebulizer	Mastro[172]	IV	LD	13	*L. pneumophila* SG3
water used for cleaning	Coetzee[176]	III	LD	2	*L. pneumophila*[d]
	Fry[177]	IV	LD	4	*L. pneumophila* SG1
dental units	Atlas[188]	IV	LD	1	*L. dumoffi*
roof-harvested rainwater	Simmons[191]	IV	LD	4	*L. pneumophila* SG1
construction & excavation	Thacker[198]	IV	LD	81	LD bacterium[d]
sullage tanks collecting bilge water	Hyland[221]	III	LD	7	*L. pneumophila*[d]
steam turbine condenser cleaning	Fraser[222]	IV	Pf	10	*L. pneumophila*[d]
inoperative bedpan flusher	Brown[173]	IV	LD	8	*L. pneumophila* SG1
steam towel warmer	Higa[223]	III	LD	1	*L. pneumophila* SG1

[a]LOEs are listed for studies that concerned outbreak investigations or case reports. [b] Legionnaires' disease/Pontiac fever. [c]Study describes multiple sporadic cases. [d]No clinical isolate was obtained.

drinking after gardening without washing hands (OR: 29.47, 95% CI: 1.96−412.14, P = 0.014) and being near dripping hanging flower pots (OR: 8.97, 95% CI: 1.41−56.96, P = 0.020). Surprisingly, the use of potting soil in the 4 weeks prior to hospitalization was not found to be a risk factor for illness.

Legionella was often detected in studies in which potting soil[43,56−60] or compost[59,61−63] were investigated. The maximum isolation rates were 91.7% for potting soil[57] and 84.8% for compost.[62] Different species of *Legionella*, including *L. pneumophila*, are regularly isolated from potting soil and compost. Several studies even found *L. pneumophila* to be predominant.[59,61−63] The maximum reported concentrations for *L. pneumophila* were 2.8×10^6 CFU/g in compost[61] and 2.8×10^4 CFU/g in potting soil.[56] Only one study reported a concentration for *L. longbeachae*, 2×10^4 CFU/g compost.[62]

Baths. A total of six studies reported on *Legionella* contamination in baths; five of these studies originated from Japan. Most studies described LD cases that were exposed to contaminated baths, and one study described the prevalence of *Legionella* in bathing facilities.

In an outbreak study, *L. pneumophila* isolates from a spa bath on a cruise ship matched a clinical isolate by PFGE, representing the highest LOE (Ia).[64] The environmental isolates were obtained from the bath water and from porous natural stones in the filters of the spa. For two cases infected by *Legionella* after using all-day-running-baths (also referred to as

DOI: 10.1021/acs.est.5b00142
Environ. Sci. Technol. 2015, 49, 4797−4815

24 h baths or ever-ready baths), a match between the patients and the environmental isolate was determined by PFGE, and other possible sources were excluded, resulting in categorization at the highest LOE.[65,66] Two studies reported neonatal LD cases associated with water birth: one was nosocomial,[67] and one involved a home water birth in an all-day-running-bath.[68] The infection source could not be confirmed because no clinical isolates were available for comparison. Baba et al.[69] isolated *L. pneumophila* from 25 out of 91 bath water samples that originated from 30 bathing facilities.

Fountains. In 13 studies, fountains were described as potential or confirmed sources. Seven studies epidemiologically linked LD cases to the fountains, and six studies investigated the presence of *Legionella* in fountains.

Fountains inside buildings, a hotel,[70] a restaurant[71] and a hospital[72] were identified as infection sources in three LD outbreak investigations. A molecular match between clinical and environmental isolates was determined in these studies by PFGE[70,72] or sequence based typing (SBT).[71] Furthermore, other possible sources were excluded, and these studies were assigned the highest LOE (Ia). Two outbreaks of Pontiac fever have been described in which indoor fountains were considered to be the probable sources.[73,74] No clinical isolates were obtained but *L. anisa* was isolated from the suspected fountains in both outbreaks, and the majority of patients had elevated antibody titers to *L. anisa*. In both studies case-control studies revealed exposure to *L. anisa* between a control group and an exposed group. In two other outbreak studies, evidence for exposure to fountains was provided, but clinical and/or environmental isolates were not obtained.[75,76] In outbreaks in which fountains are the suspected source, many patients can be involved (up to 18 LD patients[71] and 117 Pontiac fever patients,[74] see Table 3), suggesting that many people can be exposed to a *Legionella*-contaminated fountain. In environmental surveillance studies,[77−82] the detection rates of *Legionella* in fountains varied from 2.2% (3/134)[79] to 80% (4/5).[78]

Wastewater/Wastewater Treatment Plants. Wastewater or wastewater treatment plants (WWTPs) were reported as *Legionella* reservoirs or (potential) sources in 22 publications. Nine of these studies reported on WWTPs associated with outbreaks or sporadic LD or Pontiac fever cases. Thirteen of the 22 publications reported on the *Legionella* prevalence in WWTPs.

Borgen et al.[83] reported on a cluster five LD cases that were linked to a biological treatment plant at a company that produced wood-based chemicals in Norway in 2008. Clinical and environmental *L. pneumophila* isolates showed the same sequence type (ST462). Strain ST462 was isolated from an air scrubber that was the suspected infection source during a large outbreak of LD in 2005.[84] However, the outbreak strain was also found in high concentrations in the aeration ponds of the plant (10^{10} CFU/L), and Olsen et al.[85] suggested that the aeration ponds, rather than the air scrubber, were the primary disseminators of *Legionella* during the outbreaks of 2005 and 2008. Blatny et al.[86,87] studied the dissemination of *Legionella* originating from the aeration ponds. The studies surveying the biological treatment plant were categorized at the highest LOE (Ia). During an outbreak investigation concerning 86 LD cases in France, a petrochemical plant was considered the most likely infection source.[88] *L. pneumophila*, which was indistinguishable by PFGE from clinical isolates, was isolated from cooling towers, from a waste basin at the plant and in air samples collected in the vicinity of the basin, representing the highest LOE (Ia). The authors argued that both the cooling towers and waste basin played a role in the transmission of *Legionella* in this outbreak. Three studies, describing two outbreaks of Pontiac fever and two individual cases, were assigned LOE III.[89−91]

Many publications were identified that reported the detection of *Legionella* in WWTPs (see Supporting Information Table S4 for the complete set of references). Two studies investigated the prevalence of *Legionella* in a number of WWTPs and found that 27% (3/11)[92] and 59% (10/17)[93] of the plants were positive for *Legionella*. *Legionella* spp. and *L. pneumophila* were detected at different stages in the treatment process: in aeration ponds,[94,95] in WWTP influent and WWTP effluent,[95−100] and in air sampled at WWTPs.[98,101,102] One study detected *L. pneumophila* using qPCR in biosolids, which are made from sewage sludge and used for agricultural applications.[103] *Legionella* in wastewater samples were primarily detected by direct fluorescent-antibody (DFA) tests and PCR. Three studies succeeded in isolating *Legionella* from WWTP influent, effluent, or aeration ponds.[94,95,99]

Room Humidifiers. Room humidifiers were the subject of investigation in eight publications. In five studies, contaminated room humidifiers were associated with LD cases.[104−108] Three studies investigated *Legionella* contamination in humidifiers or aerosolization by humidifiers.[109−111]

Two source investigations involving infants exposed to room humidifiers filled with contaminated tap water were assigned the highest LOE (Ia).[107,108] Yiallouros et al.[107] reported a nosocomial outbreak, and Moran-Gilad et al.[108] described a community-acquired LD case. In both studies, a match was determined between the patient and environmental isolates by SBT. The detection of *Legionella* in humidifying systems was reported in two articles,[109,111] and two studies showed the aerosolization of *L. pneumophila* by humidifiers filled with contaminated water originating from hospital water systems.[110,111]

Ice/Ice Machines. A total of six studies reported on ice or ice machines as reservoirs or potential or confirmed sources of LD. In four studies, *Legionella* infection occurred through contaminated ice, and in two studies, *Legionella* was detected in ice, but no transmission to patients was reported.

To date, five cases associated with contaminated ice have been reported, all in hospital settings.[112−115] Four of the cases were suspected to have occurred through aspirated ice water, and *L. pneumophila* strains were isolated from patients and ice or ice machines.[112−114] In one case, the environmental and patient isolates were found to be indistinguishable by AFLP, and other possible infection sources were excluded, representing the highest LOE (Ia).[113] LOE Ib was assigned to the publication of Bangsborg et al.,[112] in which a match was determined based on monoclonal subtyping. The fifth patient developed LD after undergoing a bronchoscopy in which contaminated ice was used for cooling saline-filled syringes.[115] In another study, ice used for cooling saline-filled syringes was contaminated, but no patients were reported.[116] Stout et al.[117] detected *L. pneumophila* in 8 of 14 ice machines sampled in a single hospital.

Mist Machines. *Legionella* contamination of mist machines was reported in four studies. Two studies considered mist machines as the infection source, and three studies investigated the prevalence of *Legionella*. Mahoney et al.[118] reported an outbreak and also performed a prevalence study.

DOI: 10.1021/acs.est.5b00142
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Mist machines in supermarkets have been described as sources of *L. pneumophila* infection at the highest LOE (Ia, Ib) in two outbreak reports.[118,119] In both studies, *L. pneumophila* SG1 was isolated from the mist machines, and other possible sources were excluded. Clinical isolates and environmental isolates displayed the same PFGE pattern[119] or monoclonal subtype.[118] In prevalence studies, 3 of 8 mist machines in grocery stores,[118] 4 of 28 mist fans,[120] and 2 of 20 greenhouse misting machines[121] were found positive for *Legionella*.

Air Conditioning Systems. Twelve studies considered air conditioning systems. Four of these studies reported on outbreaks and one case-control study found an association between LD and home air conditioning.[122−126] Five studies reported the isolation of *Legionella* from air conditioning systems, but no patients were linked to these systems.[127−131] Air conditioners in cars have also been suggested as a potential *Legionella* source.[132,133]

Two LD outbreak investigations were included that demonstrated the highest LOE (Ib).[122,126] In these outbreaks, monoclonal antibody subtype patterns of clinical isolates matched environmental isolates from an evaporative condenser[122] and a hospital air conditioning system.[126] In the last study, the epidemic strain was also found in a cooling tower on the roof of the hospital, but the air conditioning system was considered the most likely transmission source because a fault in the design of the air conditioning system allowed for the generation of aerosols in a chiller unit of the system that served the floor in which most cases occurred.[126] In a study by Cordes et al.,[124] a contaminated evaporative condenser was the likely infection source, but no isolates were obtained from patients. One of the studies reported on the first (retrospectively recognized) Pontiac fever outbreak (1968), for which a defective air conditioning system was believed to be the infective source.[125] Although the causative agent could not be identified at the time, *L. pneumophila* SG1 was isolated from the stored lung tissue of guinea pigs that had been exposed to evaporative condenser water in 1977. In a study by Broome et al.,[123] significantly more cases were found to have home air conditioning compared to age-matched controls ($P = 0.03$). However, compared to a second control group consisting of patients with pneumonia who were seronegative for *Legionella* (IFAT ≤ 64), there was no significant difference.

L. pneumophila was detected by PCR in an LD patient who had condenser liquid leaking from the air conditioning system of his car.[132] Because *Legionella* was not isolated from the patient or the environmental sample, the evidence for the car air conditioner as an infection source was limited. Sakamoto et al.[133] detected *Legionella* DNA in evaporator compartments of the air conditioning system of 11 of 22 cars.

Natural Water: Thermal Springs. A total of 41 studies investigated hot or thermal springs. The detection of *Legionella* in thermal spring waters was described in 31 studies, and nine studies reported on cases linked to thermal spring waters. One study showed exposure to *Legionella* from thermal spring water. However, no cases were reported in this study.

For six cases, all related to Japanese spas, a genotypic match could be identified between the environmental and clinical isolates by PFGE.[136−139,141,142] In only one of the six studies, other possible infection sources were excluded; therefore, the highest LOE was assigned (Ia).[139] The other case reports were assigned the second highest LOE (II). One study reported on a case that was possibly related to a thermal spa in Switzerland.[135] A French therapeutic thermal spa was investigated in a number of studies after several LD cases were found to be epidemiologically linked to this spa.[134,140] Researchers were not able to identify the infective strains, but an association was found between the exposure of visitors and employees to the hot spring water and antibody titers of these subjects against the majority of the *Legionella* species and serotypes isolated from the spa water.[134] A similar study was conducted in a Portuguese spa in which visitors showed elevated antibody titers against the environmental *Legionella* strains.[143]

Many studies described the detection of *Legionella* in thermal spring waters that were sampled from spring sources and spa facilities (i.e., hot tubs, spas, swimming pools, wastewater)(see Supporting Information Table S5 for the complete set of references). The detection rates of *Legionella* spp. by culture ranged from 4.5%[144] to 71.9%,[145] and bacteria were detected at temperatures up to 66 °C.[146] The detection of *L. pneumophila* was reported in nearly all studies and the highest reported concentrations of *L. pneumophila* were over 10^4 CFU/L.[147] Some spring waters that were contaminated with *Legionella* were for therapeutic use.[147,148]

Natural Soil. A total of 14 publications concerned natural soil. The presence of several *Legionella* species in natural soil was described in seven publications, and seven studies considered natural soil as a potential *Legionella* source. One study provided evidence for natural soil as an infection source.

Wallis and Robinson[149] reported a single case infected with *L. pneumophila* SG1. A strain with an indistinguishable genotypic profile, determined by PFGE, was isolated from a field in which the patient had worked the week prior to illness. All other water and soil samples from the patient's home and workplace tested negative for *L. pneumophila* and LOE Ia was assigned. In 1985, Parry et al.[150] isolated *L. bozemanii* from four cases during an outbreak investigation of nosocomial LD. *L. bozemanii* was also cultured from soil in an area of excavation on hospital property and from tap water. The authors suggested that during the construction and installation of new plumbing, the plumbing system of the hospital became contaminated with *Legionella*. In another nosocomial outbreak study, soil was also considered a possible source.[151] Significantly more groundskeepers had higher serum titers against *L. pneumophila* (titer $\geq$1:128) compared to employees working indoors ($p = 0.018$). However, no *Legionella* was isolated from the soil samples or other environmental samples. In four source investigations, *Legionella* was detected in the soil; however, there was no link to the cases.[124,152−154]

Seven publications described the detection of *Legionella* in natural soil.[58,62,155−159] In Japan and Thailand, *L. pneumophila* was isolated from the soil, and the allelic profiles of some strains were identified to have been previously associated with LD cases.[156,159] One study showed the presence of *Legionella* in garden soils mixed with composted materials (6 of 14 samples).[62] The 14 garden soil samples were obtained from gardeners shown to have *Legionella* in their compost. Concentration data of *L. pneumophila* in natural soil are not available.

Cooling Liquid for Machinery. Three studies described cases linked to contaminated liquid used for cooling industrial machinery. In a plastic factory in which an LD case occurred, a machine cooling system was the suspected infection source, and a match based on monoclonal subtyping was determined between clinical and environmental isolates.[160] Other possible sources linked to the case were excluded; therefore, LOE Ib was assigned. In a large outbreak of Pontiac fever that affected 317

DOI: 10.1021/acs.est.5b00142
Environ. Sci. Technol. 2015, 49, 4797−4815

workers in an automobile plant, *L. feeleii* was found in a water-based coolant that was used to lubricate, cool, and clean the grinding and machining surfaces.[161] Ill employees had significantly higher mean antibody titers against the environmental isolate than employees that were not ill and an unexposed control group, and attack rates decreased linearly with the distance from the system. One LD case and one Pontiac fever case were linked to *L. pneumophila* SG1 found in high concentrations (1.3×10^5 CFU/L) in an uncovered water tank that acted as a heat exchange for a welding cooling system.[162] In the last two studies, no clinical isolates were obtained, but other possible sources were excluded, resulting in an LOE of III.

Other Confirmed Sources. Four studies reporting on other sources were assigned the highest LOE. In an outbreak investigation in Spain involving 11 patients, a milling machine used in street asphalt repaving was considered the infection source (LOE Ia).[163] *L. pneumophila* was isolated from the milling machine and the tank that supplied the machine with water. The water originated from a natural spring. Cayla et al.[164] reported on two patients who contracted LD after working on a cargo ship's cooling water circuit pump. *L. pneumophila* was isolated from the main valve of the pump (LOE Ia). An air-perfused footbath was the confirmed source in an outbreak report by Den Boer et al. (LOE Ia).[165] In a study by Moiraghi et al.,[166] one of the 12 suspected LD cases could be explained by the use of a contaminated underwater chest drain postoperatively after cardiac surgery (LOE Ib).

■ POTENTIAL *LEGIONELLA* SOURCES

Medical Equipment. Respiratory devices were the subject of investigation in seven publications. Five studies reported on nosocomial cases that were potentially infected by exposure to contaminated aerosols from respiratory devices.[104,105,166−168] In all investigations, *Legionella* bacteria were also detected in the hospital water system; for some studies, this could not be excluded as an infection source.[167,168] Furthermore, typing methods were not used in any of these studies to compare clinical and environmental isolates, resulting in LOEs of III or IV. The sixth publication reported the detection of *L. pneumophila* strains in air compressor systems that supply air for respirators.[169] In the last publication, Woo et al.[110] showed the aerosolization of *L. pneumophila* by respiratory equipment rinsed in contaminated tap water.

Additionally to respiratory devices, other types of medical equipment were investigated. In three studies, medical equipment was considered the most likely infection source, but the evidence was inconclusive.[170−172] Results from a case-control study conducted after an LD outbreak revealed undergoing transesophageal echocardiography (TEE) as a risk factor.[171] *L. pneumophila* SG1 strains from tap water used to rinse the TEE probes were nearly identical to clinical strains compared by PFGE. An LD case was linked to a contaminated respiratory hydrotherapy system with sulfurous spa water, but no clinical strain was available for comparison with environmental isolates.[170] Nebulizers were investigated in two outbreak studies.[172,173] Mastro and co-workers[172] demonstrated the aerosolization of *Legionella* from a medication nebulizer in an experimental setting, but the infection source remained unclear because *Legionella* was also found in other suspected sources. In a study by Brown et al.,[173] *L. micdadei* was isolated from nebulizers; however, the suspected infection source was an inoperative bedpan flusher. Medical appliances used for hydrotherapy[174] and incubator humidification trays at a neonatology ward[175] were found to be contaminated with *L. pneumophila*, but no cases were linked to these systems.

Water Used for Cleaning. Three of the selected publications reported on LD cases linked to cleaning with water in the work environment, but the evidence provided was extremely limited, resulting in low LOEs (III, IV, VI). In a report on a cluster of LD concerning two cases, an aqueous metal pretreatment tunnel in a construction equipment manufacturing plant applied to degrease and rinse steel parts was suggested as a possible infection source.[176] *L. pneumophila* was isolated from water samples from the aqueous pretreatment system, but no clinical strains were available for comparison. A case-control study was conducted after an outbreak of LD at an automotive plant, and exposure to a cleaning line was associated with LD.[177] However, no environmental isolates were obtained from the cleaning area, and strains isolated from other sites did not match with a clinical isolate. Castellani Pastoris et al.[178] reported on a patient who had been working on a drilling platform. A water gun supplied by a reservoir used for cleaning was the suspected infection source, although no environmental isolates were obtained.

Dental Units. In total, 12 studies reported on dental units as a reservoir or potential source of *Legionella*. Two studies investigated *Legionella* seroprevalence, and nine studies described the contamination of dental units by *Legionella*. Only 1 of the 12 studies selected for in this review described an LD case linked to a dental unit involving a dentist.

A culture of *L. dumoffi* was obtained from the dentist, and *L. longbeachae* and *L. pneumophila* were detected in the lung tissue by monovalent fluorescent-antibody staining.[188] All three species were also identified by monovalent fluorescent-antibody staining in samples from the dental operatory. Isolates of *Legionella* spp. were obtained from both the workplace and home but were not further typed.[188]

Consistent with the frequent contamination of dental drill units with *Legionella*, Borella et al.[189] found that dental workers had a higher *Legionella* spp. seroprevalence (IFAT, cutoff for positivity 1:128) than office staff in Bari, Italy, suggesting that dental unit water is a *Legionella* source. The widespread *L. pneumophila* colonization of eight dental stations in the London Hospital Dental Institute has been reported.[190] The *L. pneumophila* SG1 seroprevalence measured in the exposed dental staff was slightly increased compared to an unexposed group (RMAT $\geq$ 8; IFAT $\geq$ 16), but only significant with one of two used serological tests.

Legionella spp. and *L. pneumophila* are frequently detected in dental unit water samples.[179−187] In one study, *Legionella* was detected in air samples in an experimental setting by qPCR.[182] However, there was no significant difference in levels measured in the treatment room compared to background levels measured outside the treatment room.

Rainwater: Roof-Harvested Rainwater. Six studies reported the detection of *Legionella* in roof-harvested rainwater. In one study, an outbreak in New Zealand was described, involving four LD cases linked to roof-harvested rainwater.[191] *L. pneumophila* SG1 was isolated from a patient, and the same sequence type was found in roof-harvested rainwater and in a water blaster used on a nearby marina for cleaning boats. Patients were potentially infected through the use of the roof-harvested rainwater for showering, or they were infected outside by contaminated aerosols disseminated by the water

DOI: 10.1021/acs.est.5b00142
Environ. Sci. Technol. 2015, 49, 4797−4815

blaster. Aerosols spread by the water blaster may also have seeded the roof-collected rainwater systems. In five studies, *Legionella* bacteria were detected in roof-harvested rainwater, either by culture[192,193] or PCR.[194−196]

Construction and Excavation. Construction and excavation sites were occasionally considered as possible infection sources in older publications.[197−199] However, evidence for construction and excavation sites as sources of *Legionella* was hardly provided. In 1978, a nosocomial outbreak of LD in 1965 was studied, and soil from an excavation site near the hospital was suggested as the potential reservoir of the infectious agent based on an epidemiological investigation.[198] Sleeping by open windows and having free access to the grounds of the hospital were identified as risk factors. The results from a case-control study conducted by Storch et al.[197] implied that sporadic LD patients were more likely to have lived near excavation sites than controls. Furthermore, there was a significant excess of construction workers among the patients. In a Spanish study, a significantly higher *L. pneumophila* antibody prevalence was measured (IFAT, titer ≥ 1:64) in a group of 87 underground construction workers compared to a group of 150 healthy blood donors (16% and 1.2%, respectively).[199] It should be noted that the mean age of the blood donors was slightly lower than that of the construction workers. Furthermore, the group of workers consisted of all men, in contrast to the group of blood donors, which was comprised of 80 men and 70 women.

Natural Water: Surface Water and Groundwater. Surface water was the subject of investigation in 36 publications. In 34 publications, the presence of *Legionella* in surface water was described and two case-control studies found an association between LD incidence and surface water use. Nine publications concerned groundwater.

Den Boer et al.[200] investigated whether a geographical variation in LD incidence in The Netherlands coincided with geographical differences in the origin of drinking water (groundwater versus surface water). The price of water was used as a proxy because higher prices result from more intensive production processes using raw surface water. The results showed that a high water price was positively associated with a high LD incidence rate at the municipal level (OR: 5.1, 95% CI: 3.3 to 8.0). Ng et al.[201] discovered an association between the incidence of LD in the Greater Toronto Area, Canada, and lower river and creek levels and decreases in the temperature of Lake Ontario (the drinking water source for most residents). The authors hypothesized that low water levels might promote the growth and survival of *L. pneumophila* in treated or untreated water supplies, and cooling of the lake might influence LD risk via increased lake circulation.

Many selected publications described *Legionella* detection in surface water (see Supporting Information Table S6 for the complete set of references). In 1981, Fliermans et al.[202] demonstrated the ubiquity of *L. pneumophila* in surface waters by investigating 67 lakes and rivers in the U.S. The study showed that *L. pneumophila* was able to survive a wide range of psychical and chemical conditions (e.g., temperature, pH). Several other studies have found *Legionella* in natural waters with extremely diverse characteristics, for example, a lake in Antarctica,[203] rivers in a Brazilian rainforest,[204] an acidic geothermal stream in the U.S.,[205] hydrothermal lakes in Europe,[206,207] and marine and estuarine environments in the USA, Puerto Rico and Brazil.[99,204,208−210] Two outbreak strains involved in outbreaks related to a WWTP in Norway (see 'Wastewater/Wastewater Treatment Plants') were isolated from a river downstream of the outlet of the plant, whereas they were not detected upstream of the outlet.[85] The authors hypothesized that the river played a role in the dissemination of *L. pneumophila* during the outbreaks. The maximum reported concentration of *L. pneumophila* in surface water, 1.9 × 10^6 CFU/L, was reported in this study.

Nine studies described *Legionella* prevalence in groundwater.[174,211−218] The maximum reported concentration of *L. pneumophila* in groundwater was 8 × 10^2 CFU/L.[211]

Rainwater: Rainwater on the Road. *Legionella* presence in rainwater on the road was shown in three studies. In a study investigating puddles at six locations on asphalt roads in Japan, 47.8% of 69 samples were positive for *Legionella* species.[219] Strains of *L. pneumophila* SG1 were the most frequently isolated. Sakamoto et al.[220] found a relation between the prevalence of *Legionella* in rainwater puddles on roads in Japan and ambient temperature on the sampling date; the isolation rate of *L. pneumophila* increased from 15.8% at temperatures below 20 °C to 58.3% at temperatures above 25 °C. In The Netherlands, *L. pneumophila* bacteria were isolated by amoebal coculture from pluvial floods after intense rainfall (3 of 6 samples).[95] Two strains belonged to sequence types that had been previously identified in patients. Whether human exposure to rainwater on roads poses a risk for *Legionella* infection and possible LD remains unclear.

Other Potential Sources. In 23 publications, potential sources or reservoirs of *Legionella* were described that were only mentioned in one or two publications selected in this review. The following potential sources were considered in source investigations: discharge vessels of sullage tanks collecting bilge water from ships,[221] a steam turbine condenser,[222] an inoperative bedpan flusher,[173] a steam towel warmer,[223] an industrial air scrubber located at a WWTP (see Wastewater/Wastewater Treatment Plants),[83,84] and garden hoses.[36,224] Low LOEs (III−V) were assigned because the evidence was inconclusive or another suspected source was found to be the most likely source.

Furthermore, *Legionella* bacteria were isolated from the following potential sources/reservoirs: indoor pools,[225] eyewash stations,[226] bracts of rainforest epiphytic plants,[209] telephone manholes,[227] water and filters from a water-cooler,[228] a material reclamation facility,[229] machines used for street cleaning,[163] car wash stations,[88] and a steam discharge pipe extending from a boiler.[230] *Legionella* bacteria were detected by molecular methods in the following potential sources/reservoirs: aquarium water,[231] slow sand filters used in horticulture,[232] coral,[233] acid mine drainage[234] and bottled mineral water.[235] Shahamat et al.[129] tested several potential sources of *Legionella* on a university campus and detected *Legionella* by DFA in a steam tunnel, a chiller, air handling units, sump pits and expansion tanks.

Legionnaires' Disease Risk Factors. A total of 12 epidemiological studies identified risk factors for infection with *Legionella* and reported diving, exposure to industrial aerosols, factors related to driving, and meteorological factors. Neubauer et al.[236] showed that divers had a significantly higher prevalence of positive antibody titers (titer ≥1:64) than controls. The divers were significantly older than the controls, and no information other than age was provided regarding the controls. In a geographical ecological study by Che et al.,[237] an association was found between exposure to industrial aerosols and the incidence of sporadic community-acquired LD cases in France. Two studies identified that professional drivers were at

DOI: 10.1021/acs.est.5b00142
Environ. Sci. Technol. 2015, 49, 4797−4815

increased risk of acquiring LD.[238,239] In the study by Wallensten et al.,[239] two risk exposures in relation to driving were identified: 'driving through industrial areas' (OR: 7.2, 95% CI: 1.5−33.7) and 'using no screenwash in the windscreen fluid' (OR: 47.2, 95% CI: 3.7−603.6). The publication also discussed isolating *Legionella* from windscreen fluid not containing screenwash of one car in a pilot study, but the data were not presented. Several studies have investigated the association between meteorological variables and LD incidence in Europe (The Netherlands, Spain, Switzerland, and the UK), the U.S., and Canada. Temperature (either average or short-term),[240−244] relative humidity (or vapor pressure),[201,240,242−244] and precipitation[242,243,245,246] were identified as meteorological variables that are associated with LD incidence.

DISCUSSION

This review shows that many different systems and matrices could be sources of *Legionella*. A total of 17 systems and matrices were classified as confirmed sources of *Legionella* (LOE I assigned to at least one study). For cooling towers, whirlpools, potting soil/compost, baths, fountains, wastewater/WWTPs, ice/ice machines, room humidifiers, mist machines, air conditioning systems, thermal springs, natural soil, a milling machine, a ship water pump, a foot bath, a chest drain, and cooling liquid for machinery, matches have been made between *Legionella* isolates from these systems and clinical isolates. Furthermore, for all of these sources, there was additional evidence provided that these sources were the infection source, such as evidence of the exposure of several cases to the same source or evidence of *Legionella* spread from the source. Several of these sources were confirmed sources in large outbreaks with five or more cases. This concerned not only well-known sources, such as cooling towers, whirlpools, air conditioning systems, potting soil/compost, and fountains but also wastewater/WWTPs, room humidifiers, mist machines, and a milling machine. The evidence for some sources was derived from one or two studies, such as mist machines, whereas other sources, that is, cooling towers, whirlpools, and potting soil/compost, were confirmed sources in at least four source investigations.

There were no systems or matrices where the highest LOE assigned was LOE II. LOE III was assigned to the following potential sources: TEE probes, a hydrotherapy system, water used for cleaning, and sullage tanks collecting bilge water. Besides the before mentioned, *Legionella* has been found in many other man-made systems or natural matrices, but either no patients were linked to these reservoirs or the supporting evidence was weak, resulting in a low LOE (LOE IV−VI). However, these systems or matrices could play an important role in the transmission of infectious *Legionella* bacteria; they might not yet be considered in source investigations, resulting in an underestimation of their importance.

To compare environmental isolates and patient isolates, advanced methods currently available for typing *Legionella* should be used in addition to serotyping. In this review, molecular typing (AFLP, PFGE, SBT) was assumed to provide the best evidence that the *Legionella* bacteria isolated from a patient and from a suspected source belonged to the same strain.[247] However, not all methods have the same discriminatory power. The best discriminatory method is whole genome sequencing (WGS) of *Legionella* isolates. This has only recently been conducted in a few LD outbreak investigations.[248−250] Sánchez-Busó et al.[251] retrospectively performed WGS on clinical and environmental *L. pneumophila* strains linked to 13 outbreaks, and in some occasions the genome sequences of isolates of the same sequence type and outbreak did not cluster together and were more closely related to sequences from different outbreaks. This suggests that, for some sequence types, the current SBT approach provides insufficient resolution to establish the outbreak source. Definitive proof that a potential source was the infection source is difficult to obtain. The combination of evidence is necessary to confirm the source.

Potting soil and compost as a reservoir and source of *L. longbeachae* have been described in studies from many countries. A vast proportion of LD cases are reported to be caused by *L. longbeachae* in Australia and New Zealand;[10,11] however, in Europe and the U.S., *L. pneumophila* is dominant.[7−9] Recently, it was also suggested that a certain proportion of LD cases in Europe are caused by *L. longbeachae.*[252] Some studies identified *L. pneumophila* as the predominant species in potting soil and compost over *L. longbeachae.*[56,62] However, to our knowledge, no LD cases caused by *L. pneumophila* from potting soil or compost have been reported to date. Perhaps potting soil and compost are not yet considered potential sources of *L. pneumophila* in source investigations; therefore, it is important to assess whether *L. pneumophila* originating from potting soil or compost can cause disease.

Legionella spp. and *L. pneumophila* are also present in natural soil. However, only one study provided evidence for infection caused by *L. pneumophila* in natural soil.[149] The patient worked at a nursery and had spent several days potting plants on a field. Possibly, the soil that harbored the infective strain, obtained from this field, was a mixture of natural soil and potting soil. In certain professions, exposure to natural soil might be substantial;[151,199] to assess the possible infection risk for *Legionella* originating from natural soil, concentration data should be determined and the virulence of soil-borne *Legionella* strains should be assessed.

Surface water, groundwater and rain are reservoirs of *Legionella* but evidence that these matrices are sources of infection is lacking. Data on aerosolization of *Legionella* from these matrices and subsequent exposure of humans, for example, during heavy rain events or when surface water is used for cleaning purposes, could give insight in the possible risk that these reservoirs of *Legionella* pose to human health. River water has been described as a possible *L. pneumophila* source in two studies in which patients contracted LD after nearly drowning.[37,253] However, for both cases, *Legionella* was not detected in the river water. Based on the study inclusion criteria these studies were excluded from this review.

Only one case linked to dental treatment was identified in this review, and the evidence for the dental unit as the infection source was inconclusive.[188] A recent study described a case of an 82-year-old woman who died of LD after dental treatment.[254] This study was not identified in our literature search because the main search terms ['legionell*', 'legionnair*', "Pontiac fever"] were not included in the title, and there was no abstract. This publication would have been assigned the highest LOE (Ia). It is surprising that not more cases are linked to dental units because dental units are often found to be colonized by *Legionella*, and risk groups are expected to visit dentists. Dental units may not always be targeted in outbreak investigations.

DOI: 10.1021/acs.est.5b00142
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Several risk factors for acquiring LD are reported in the literature that might give insight into yet unknown sources. Further study is necessary to unravel the meaning of the risk factor 'being a driver as a profession'.[238,239] Furthermore, although the weather likely plays a role in the growth and transmission of *Legionella*, which sources or reservoirs of *Legionella* are under the influence of the weather and how weather conditions contribute to the growth and transmission of *Legionella* remains unclear.

After the literature search was conducted, some studies were published that reported on (potential) sources of *Legionella* that were not represented in this review or that supplied information that significantly changed the LOE assigned to a potential source. Euser et al.[255] identified a manually operated pressure test pump as the infection source for a sporadic LD case. Environmental and clinical isolates were matched by SBT. Wei et al.[256] found that *Legionella* strains from water dispensers used to make infant formula were indistinguishable by SBT from strains obtained from two nosocomial neonatal cases. Litwin et al.[257] linked an LD case to a contaminated recreational vehicle water reservoir. In a Greek study, *Legionella* was detected in car cabin air filters.[258] Although *Legionella* was already detected in car wash stations,[88] a recent publication provided essential evidence for these stations as a *Legionella* source.[17] One study provided evidence for the possible transmission mode of *Legionella* originating from compost by demonstrating that compost could release bioaerosols containing *Legionella*.[259]

This review has several methodical limitations. Relevant publications on potential sources of *Legionella* might have been missed because this review focused on publications published in biomedical journals because of the use of a single search engine: PubMed. However, in every publication selected for full-text assessment, the Introduction and Discussion sections were checked for relevant references that were missed in this review. It was concluded that literature regarding *Legionella* in dental units was missed in our search. Another search was conducted using two different search engines, but no other publications were found that supplied information that significantly changed the assigned LOE. Furthermore, the selection criteria may have excluded relevant publications on (potential) sources of *Legionella*. For example, studies on travel-related LD were excluded because it was assumed that most patients would be infected by the use of contaminated tap water through showering, possibly resulting in a loss of publications about other (potential) sources. And by excluding comments, a study describing the isolation of *L. pneumophila* from windscreen washer fluid was missed.[260] Several publications on thermal springs as possible sources of *Legionella* were missed because these studies were published in Japanese.[261−266] The inclusion of the missed publications would have likely resulted in more studies providing the highest LOE.

In designing the LOEs used in this review, *Legionella* concentration data were not taken into account. Concentration data could be used to assess the (relative) infection risk for a (potential) source. For example, Ahmed et al.[196] calculated that the concentration of *Legionella* in roof-harvested rainwater, as determined by PCR, was such that the infection risk associated with the use of rainwater for showering and garden hosing was to be well below the threshold value of one additional infection per 10,000 persons per year. However, data on concentrations are not always valuable depending on the type of detection method used. Molecular methods could overestimate the level of contamination and detection by culture could lead to false negative results.[267]

This review demonstrates that many different water systems and nonwater systems are reservoirs of *Legionella* and many different systems and matrices have been confirmed as sources of *Legionella*. LD can be acquired by exposure to relatively rare sources that may not yet be considered in source investigations. Therefore, when tracking a source of infection it is essential to consider all possible potential and confirmed sources. Further research is needed to unravel what the contribution is of each confirmed source, and possibly also potential sources, to the LD disease burden. The knowledge about sources of *Legionella* is imperative when developing policy for effective *Legionella* prevention and control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The full text of the Methods section, Figure S1, and Tables S4−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 0031-30-2743692; e-mail: Eri.van.Heijnsbergen@rivm.nl.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was performed on behalf of and for the account of the Ministry of Health, Welfare and Sport (V/210321). We thank J.P. Ridder-Kools for her assistance with the literature search.

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DOI: 10.1021/acs.est.5b00142
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DOI: 10.1021/acs.est.5b00142
Environ. Sci. Technol. 2015, 49, 4797−4815