Currie, Sandra L. and Beattie, Tara K. (2015) Compost and Legionella longbeachae : an emerging infection? Perspectives in Public Health, 135 (6). pp. 309-315. ISSN 1757-9139 , http://dx.doi.org/10.1177/1757913915611162

Intended category: Review

Title: Compost and *Legionella longbeachae* – An emerging infection?

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Abstract

Human disease caused by *Legionella* species is dominated by *Legionella pneumophila*, the main causative agent in cases of Legionnaires' disease. However other species are known to cause infection, e.g. *Legionella longbeachae* causes an equivalent number of cases of disease as *L. pneumophila* in Australia and New Zealand. Infection with *L. longbeachae* is commonly associated with exposure to composts and potting soils, and cases of infection with this organism have been increasing in Europe over the past 10 years. The increase in incidence may be linked to factors such as increased awareness of clinical presentation, or due to changing formulation of growing media, although it should be noted that the presence of *Legionella* species in growing media does not correlate with the number of cases currently seen. This is likely due to the variables associated with infection, for example, host factors such as smoking or underlying health conditions, or difference in growing media storage or climate, especially warm humid conditions, which may affect survival and growth of these organisms in the growing media environment. There are numerous unknowns in this area and collaboration between growing media manufacturers and researchers, as well as more awareness among diagnosing clinicians, laboratory staff and the general public is necessary to reduce risk. More research is needed before definitive conclusions can be drawn: *L. pneumophila* research currently dominates the field and it is likely that the overreliance on diagnostic techniques such as the Urinary Antigen Test which is specific for *L. pneumophila* Sg 1, is detrimental to the diagnosis of *L. longbeachae* infection.

Introduction

The term Legionnaires' disease was first coined in 1976, after 182 attendees at a meeting of the American Legion showed symptoms of a mystery illness. It took a further 6 months before the causative agent in the outbreak, a Gram negative rod, was identified and named *Legionella pneumophila* (1). Since then, over 50 species of *Legionella* have been identified, with 64 serotypes.

Despite the numerous pathogenic species found, *L. pneumophila* is the main causative agent of human disease. An international-collaborative study by Yu and colleagues, comparing the most common causative agents in cases of sporadic community-acquired legionellosis from the USA (72.2% of cases reviewed), Italy (12.6%), Switzerland (6.1%), New Zealand (4.3%) and Australia (4.7%), cited *L. pneumophila* as the agent responsible for 91.5% of cases of Legionellosis; the second most commonly isolated species, *Legionella longbeachae*, was present in 3.9% of cases (2). Despite the low representation worldwide, *L. longbeachae* plays a significant role in the burden of legionellosis in the southern hemisphere; in the study by Yu, 14 of the 20 *L. longbeachae* isolates came from Australia and New Zealand (2). A review of legionellosis survey data in Southern Australia from 1996 to 2000 reported that 42% of cases were attributable to *L. longbeachae*, compared with 51% due to *L. pneumophila* (3). In Western Australia, between 1999 and 2010, 87% of diagnosed cases of Legionnaires' disease were caused by *L. longbeachae*, whereas only 9% of cases were caused by *L. pneumophila* (4). Similarly, in New Zealand, the Ministry of Health found that, in 2011, *L. longbeachae* was responsible for more cases than *L. pneumophila*, with 42% and 30% of laboratory-reported cases of infection, respectively (5). Human infection with *L. longbeachae* has also been noted in the USA (6), Japan (7) and Thailand, where Phares et al. found that *L. longbeachae* was responsible for 5% of clinically defined cases of pneumonia in a rural district, whereas *L. pneumophila* was not reported (8).

Historically, the incidence of infection with *L. longbeachae* in Europe has been low; however, as noted by Whiley and Bentham, the number of cases of infection appears to be increasing (9). In 2012, Lindsay et al noted that *L. longbeachae* had been cited as the causative agent in only 11 cases of infection in the UK since 1984, seven of these occurred in Scotland (10). Further work revealed that between 2008 to date, 26 cases of *L. longbeachae* infection had been detected in Scotland; in most cases the patient had been in contact with commercially available growing media before the onset of symptoms (11) (personal communication, Dr Kevin Pollock/Ross Campbell, Health Protection Scotland). In addition, an atypical case of *L. longbeachae* cutaneous infection in a female patient in the UK was recently described (12).

This study presents a review of the literature currently available examining *Legionella* spp in the compost habitat, particularly the status of *L. longbeachae* infection. Work was completed using Web of Knowledge and PubMed searches including, but not limited to, individual and combinations of the following terms: *Legionella,* Legionnaires disease, Pontiac fever, *Legionella longbeachae,* soil, compost, growing media, garden, amoeba, PCR, diagnosis, biofilm, source, water. Searches were not truncated by date, although the relatively recent discovery of this organism reduces the amount of searchable literature available.

Infection

Infection with *Legionella* spp can be symptomatic or asymptomatic: hospital patients and healthy individuals have been shown to experience increased antibody titres to *Legionella* antigens without showing clinical signs of infection (13, 14). Symptomatic infection will generally present as legionellosis in one of two distinct clinical manifestations: Pontiac fever (PF)—a self-limiting influenza-like illness; or Legionnaires' disease (LD)—a more serious pneumonia that can be fatal. However there have also been a number of atypical manifestations of *Legionella* infection, e.g. cutaneous infection (15) caused by *L.*

pneumophila Sg 8, prosthetic joint infection caused by *L. micdadei* (16) and a septic foot infection (17) and endocarditis (18) both caused by *L. longbeachae*.

A number of symptoms, appearing after a 2-10 day incubation period, are associated with LD, including malaise, shortness of breath, fever and diarrhoea; this is the most serious form of the disease and, on average, is fatal in 10% of cases (19). Cases of infection can be community, nosocomial or travel-related; in 2013 in the UK, the majority of cases were either community acquired (179 of 331) or travel-related (148 of 331) (20). Pontiac fever is a less serious manifestation of infection, with flu-like symptoms appearing 1-2 days after exposure, and resolving without intervention within a week (21). Unlike LD, where both immunosuppression and increased age are risk factors for infection, PF does not appear to discriminate between adults and children, healthy or immunocompromised individuals (22). Indeed, exposure to a PF source is more likely to result in illness than exposure to a LD source. Information for clinicians from the Centers for Disease Control and Prevention (CDC) shows that when exposed to the source of LD, <5% individuals become ill, compared with >90% of those exposed to the source of PF (23). The reason why exposure to *Legionella* spp results in different clinical manifestation remains unclear. Rowbotham (24) suggested that pathogenesis of LD is caused by the invasion and replication of *Legionella* bacteria within human cells, whereas PF is due to hypersensitivity caused by an unknown component of the bacteria or an amoebal host.

Although around 40-50% of identified *Legionella* spp have been shown as agents of human disease including *L. pneumophila*, *L. longbeachae, L. bozemanii, L.micdadei* and *L. anisa* (25, 26), many of these are identified rarely in clinical samples, and others have only been identified once. There does not appear to be a difference between species of *Legionella* and their ability to cause PF; *L. pneumophila* and *L. longbeachae* have both been responsible agents in outbreaks (27, 28). Comparisons between the

clinical presentation of LD caused by *L. pneumophila* and *L. longbeachae* showed no significant difference in symptoms observed between the two species (29).

When comparing the genomes of *L. pneumophila* and *L. longbeachae* there are a number of similarities, perhaps indicating why these are the most successful pathogens in the *Legionella* genus. Gomez-Valero et al found 124 genes specific to *L. pneumophila* and *L. longbeachae* which "increase successful infection of mammalian cells" when comparing their genomes with those of *Legionella micdadei, Legionella hackeliae* and *Legionella fallonii* (a *Legionella*-like amoebal pathogen designated LLAP-10), species much less likely to cause disease in humans (30). However, unlike *L. pneumophila, L. longbeachae* lacks flagella and produces a capsule (31), which along with a chemotaxis system and sequences for cellulolytic enzymes in the *L. longbeachae* genome, but not the *L. pneumophila* genome, is likely to help its survival, for example, in the potting soil environment, and from host defences (31). While *L. longbeachae* appear to have adapted to soil life, it is also likely that *Legionellae* survival in compost and the composting process is aided by an association with soil-dwelling free living amoebal host species, which may provide a niche habitat away from the potentially harmful environment. Such protective symbiosis has been noted before, for example, *Acanthamoeba* spp, which are often used for co-culture work (32), can both protect and revive *L. pneumophila* after treatment with sodium hypochlorite (33). It should be noted though that limited work has investigated such symbiotic relationships for *L. longbeachae*.

Diagnosis and Treatment

Fast accurate diagnosis is key to successful treatment of disease. There are numerous techniques available for the diagnosis of *Legionella* spp infection, including the urinary antigen test (UAT), serological testing, PCR and culture from patient samples. Culture on buffered charcoal yeast extract agar (BCYE) is seen as the "gold-standard" in identification of *Legionella* spp; however, colony growth

110 can take 3-10 days which is much slower than other available methods and is undesirable in a clinical
111 setting where fast diagnosis is preferred (34). This is likely one of the reasons why 79% (5162/6601) of
112 cases in Europe in 2013 were identified by the UAT compared with only 11% (720/6601) identified by
113 culture (20). The UAT is only specific for *L*. *pneumophila* Sg 1 and may be a contributing factor in the late
114 diagnosis of infections caused by non-Sg1 *L. pneumophila* and other species of *Legionella*. In addition,
115 Thalanayar et al. showed that the urine test is not accurate in all cases; these authors found a negative
116 result when serum levels showed a positive reaction to *L. pneumophila* Sg1 and elevation from 1:64 to
117 1:1024 (35). Cases *of L. longbeachae* infections have been seen in Australia since 1989 (36) and it is likely
118 that this species is tested for more widely here than in the Northern Hemisphere due to increased
119 awareness amongst clinicians. Likewise, the recent increase in *L. longbeachae* infection seen across
120 Europe may also be linked to increased clinical awareness following media reports highlighting patient
121 case studies, clusters and research in this field. As well as incorrect or slow diagnosis of LD, the self-
122 limiting nature of PF means that it is unlikely to be properly diagnosed unless an outbreak occurs (37).

123 A cluster of *L. longbeachae* infection occurred in Scotland during summertime 2013 and four out of six
124 of these cases were initially identified using PCR in the NHS Lothian region; the diagnostic lab had
125 implemented *Legionella* spp PCR testing for all severe community acquired pneumonia (CAP) patients in
126 2010 (11). Work by Murdoch et al suggests that PCR diagnosis using primers targeting a *Legionella*
127 specific region of the 16S rDNA gene may be more effective even than the preferred culture method
128 (38). When comparing data on Legionellosis two years before and two years after the introduction of
129 PCR testing for *Legionella* spp on all respiratory specimens, the authors found a fourfold increase in
130 diagnosis of *Legionella* spp infection when moving from culture to PCR diagnosis (38). PCR is suitable in
131 the relatively fast identification of *Legionella* spp, and does not have the species limitations of the UAT.

The British Thoracic Society recommends the use of this technique over serological testing where available (39). Use of this method as a preliminary identification technique prior to the culture of samples for typing and confirmation may be beneficial in faster diagnosis of this disease in the future. In the UK, pneumonia affects up to 11 in 1,000 adults each year (40) and can be caused by a number of different bacteria, viruses and fungi. Lamoth and Greub noted while reviewing literature on respiratory tract infections that the aetological agent in 50% of CAP and 75% of nosocomial pneumonia remains unknown and it is possible that a change in diagnostic practice could lead to identification of more cases than currently observed (41).

Due to the slow nature of such diagnosis it is possible that the correct antibiotic regimen may not be administered in a timely fashion leading to poorer patient outcomes, extended hospital stays and inevitably escalating treatment costs. In addition, resistance of a variety of bacteria to all classes of antibiotics has been seen to be increasing over time. The removal of the sources of infection is preferable to overreliance on antibiotics for treatment, leaving drugs for patients who are most seriously infected.

Source

L. longbeachae was first isolated in 1981, from a clinical sample taken from a patient with CAP (42). Subsequent cases of LD where *L. longbeachae* was the aetiological agent have been widely linked to gardening (10, 43, 28); cases reported range in severity from an outbreak of PF (28) to LD requiring treatment in an intensive care unit (ICU) (11). The link between gardening and *L. longbeachae* was first made by Steele et al, who isolated the organism from potting soils in South Australia after an outbreak affecting 23 people identified gardening as a major risk factor for infection (44). Since then, *L. longbeachae* has been isolated from compost and potting mixes in Japan (45), Switzerland (46), Greece

(47), Scotland (48), and the USA (6), but has not been isolated from water, unlike other species of *Legionella*.

The high microbial diversity in growing media means that *Legionella* can be difficult to culture due to inhibition by other organisms, plate overgrowth and insufficient agar media, and it may be the case that sources of infection, other than water, have been overlooked in the past due to this fastidious nature of *Legionella* spp. Increased identification of *Legionella* from this environment may also be due to the changing composition of composts, for example, the reduction of peat content in the UK (49). It is possible that variety in compost composition affects the conditions and subsequently different species survival in growing media. Steele et al (50) isolated *Legionella* including *L. longbeachae* from potting composts and green wastes, but not from peat alone. Two similar studies did not isolate *Legionella* spp from 100% peat samples (45, 47). A report for the South Australian guidance committee noted that the source of *Legionella* spp in compost is inconclusive, but that plants and trees may be a source of these organisms (51). It is important to note that *L. longbeachae* was not isolated in a study looking at the prevalence of *Legionellae* at compost making facilities and green waste storage plants in Switzerland (52) but was isolated in a more recent Swiss study (53). Differences in detection may be due to the high limit of detection of *Legionella* spp from environmental samples and subsequent growth during the composting process.

Often the source of sporadic Legionellosis infection is not discovered. Of six cases of *L. longbeachae* infection identified in Taiwan 2006-2010, only two identified specific soil exposure (54). In addition, composts and soils should not be ruled out as a source of infection for other species of *Legionella*. *L. pneumophila* Sg1 has been isolated from compost and soil samples (46-48, 55), and Wallis and Robinson

associated a case of *L. pneumophila* infection with soil (56). *L. pneumophila* pneumonia described by Thacker et al was also thought to have soil as a source (57).

Transmission

The main route of transmission for LD is widely regarded as through the inhalation or aspiration of water aerosols contaminated with *L. pneumophila* (26). For infection linked to compost use, there is more debate. There have been suggestions that *Legionella* spp may be able to enter the body through open abrasions in the skin (58, 59), while Steele et al suggested that *L. longbeachae* leaches out of potting mix after watering, and may be present in any aerosols formed during the watering process, which could be inhaled by the gardener (44). Work by Doyle et al found that an aerosolized Australian clinical isolate of *L. longbeachae* Sg1 was lethal to 3 out of 5 exposed Guinea pigs, and lung tissue showed similar characteristics to infection with *L. pneumophila* Sg1 upon post mortem examination (60), suggesting that aerosolization would be a viable route of infection.

Inhalation or aspiration of live bacterial cells, contaminated dust or soil particles (61, 62), or protozoa containing the bacteria (63) are also potential routes of infection. Rowbotham suggested that amoebae or vesicles released from amoebae could prevent dehydration of legionellae and through inhalation could provide a large dose of the bacteria to a potential host (64). Work by Cabello-Vílchez et al provides support for this theory as *Acanthamoeba* spp were isolated from 21 (28.4%) of 74 nasal swabs taken from healthy individuals in Peru (65), and another study found amoebae-resisting bacteria after amoebal co-culture of human nasal swabs (7 out of 444 samples)(66). Berk et al also described the release of respirable vesicles containing live clusters of *L. pneumophila* by *Acanthamoeba polyphaga* and *Acanthamoeba castellanii* (67). Although Cramp et al described a cluster of PF attributed to aerosolized potting mix, the source of infection remains unclear, as contaminated soil, dust, water,

protozoa and bacteria may all have been present in the air (28). However, this does support the theory that the inhalation of aerosols consisting of contaminated water or compost particles is the most likely route of infection, as does the evidence that this is the method for transmission of *Legionella* spp found in water. Proximity to dripping hanging flower pots was found to be a predictor for infection and aerosolization suggested as a likely mode of transmission (68). Conza et al isolated *L. pneumophila* and Free-living amoeba (FLA) from 10.6% (5/47) and 19.1% (9/47) of bioaerosol samples, respectively, collected at composting facilities; however the authors did not isolate *L. pneumophila* and FLA simultaneously from the same samples, including potential intracellular *Legionella* spp (53). The evidence suggests that transmission occurs when live *Legionella* spp, or contaminated compost particles or water droplets are aerosolized when the growing media is handled, when bags are opened or when the material is watered.

Risk Factors

The number of cases of *L. longbeachae* reported does not tally with the frequency with which the organism is isolated from growing media. The potential for greenhouse storage to increase levels of legionellae in growing media was noted, based on observations of amoebal enrichment and a preliminary study, by Lindsay et al (10). A limited increase of legionellae was seen in some but not all amoebal enrichment studies (45, 48) which may suggest that these species increase in numbers in warm humid conditions, for example as could be provided in a greenhouse. Recent work examining a cluster of six *L. longbeachae* infections did not identify a common growing media product or manufacturer, but did isolate the organism in growing media from 5 out of 6 cases (11). It was noted that growing media had been stored inside the house, greenhouse, car, polytunnel, shed or garage of the infected individuals. This, combined with the higher than normal temperatures seen in Scotland during the time

219 that this cluster occurred, leads the authors to suggest that climatic conditions and storage of the
220 growing media may have enabled high levels of growth, leading to increased risk of human infection. An
221 analysis of 1676 community-acquired cases in England and Wales between 1993 – 2008 identified a
222 higher risk of sporadic LD after warm wet weather (69), and wet, warm and humid weather was linked
223 to the occurrence of legionellosis in metropolitan Philadelphia between 1995-2003 (70).

224 O'Connor et al highlights that presence of *Legionella* spp in growing media does not necessarily indicate
225 that those handling it will become infected, and also that education of potential risk factors and hand
226 washing before eating, drinking and smoking was shown to decrease incidence of infection (68).

227 **Conclusions**

228 While *L. pneumophila* Sg1 is the main causative agent of LD, *L. longbeachae* is responsible for a
229 significant burden of legionellosis infection in the southern hemisphere, particularly Australia and New
230 Zealand; in animal studies, Australian strains of *L. longbeachae* were more virulent than strains from
231 elsewhere (71), which may help to account for the discrepancy in infection rate between Australia and
232 other countries. However there has been an apparent increase in cases of infection caused by *L.*
233 *longbeachae* in UK in the last ten years. This may be linked to factors such as increased awareness of
234 clinical presentation, or due to changing formulation of growing media. *Legionella* spp were isolated
235 from 62.5% (15/24) of UK compost samples (48), but the prevalence in compost does not correlate with
236 number of cases currently seen. Both *L. pneumophila* and *L. longbeachae* may be adapted to infect
237 mammalian cells better than other species (30), however as these species are not the most commonly
238 found in growing media, the potential for infection via this route is low, i.e. the presence of *Legionella*
239 spp in growing media may not be indicative of the risk of infection.

240 The variables involved in *Legionella* related infection linked to compost are summarised in Figure 1.
241 More research is needed before definitive conclusions can be drawn. *L. pneumophila* research
242 dominates the field; a crude search using ISI Web of Science gives 369 hits and 29,632 hits when
243 searching for "*longbeachae*" and "*pneumophila*" respectively. There are numerous unknowns in this
244 area and collaboration between growing media manufacturers and researchers, as well as more
245 awareness among diagnosing clinicians, laboratory staff and the general public, is necessary. It is likely
246 that specific conditions are needed before infection occurs, including: host factors such as smoking or
247 underlying health conditions; storage and climate, especially warm humid conditions; transmission of
248 infective agent through method of compost use; and presence or absence of pathogenic strains and
249 their host species in the growing media environment, which may be impacted by composition. McDade
250 highlights the importance that recognition and pursuit of anomalies in routine investigation plays in new
251 discoveries, and the potential pitfalls of sticking to a standard diagnostic algorithm (72). This may well
252 be true of the current system: while the importance of *L. pneumophila* Sg1 as an aetiological agent
253 cannot be denied, it is likely that the overreliance on the Urinary Antigen Test is detrimental to the
254 diagnosis of *L. longbeachae* infection.
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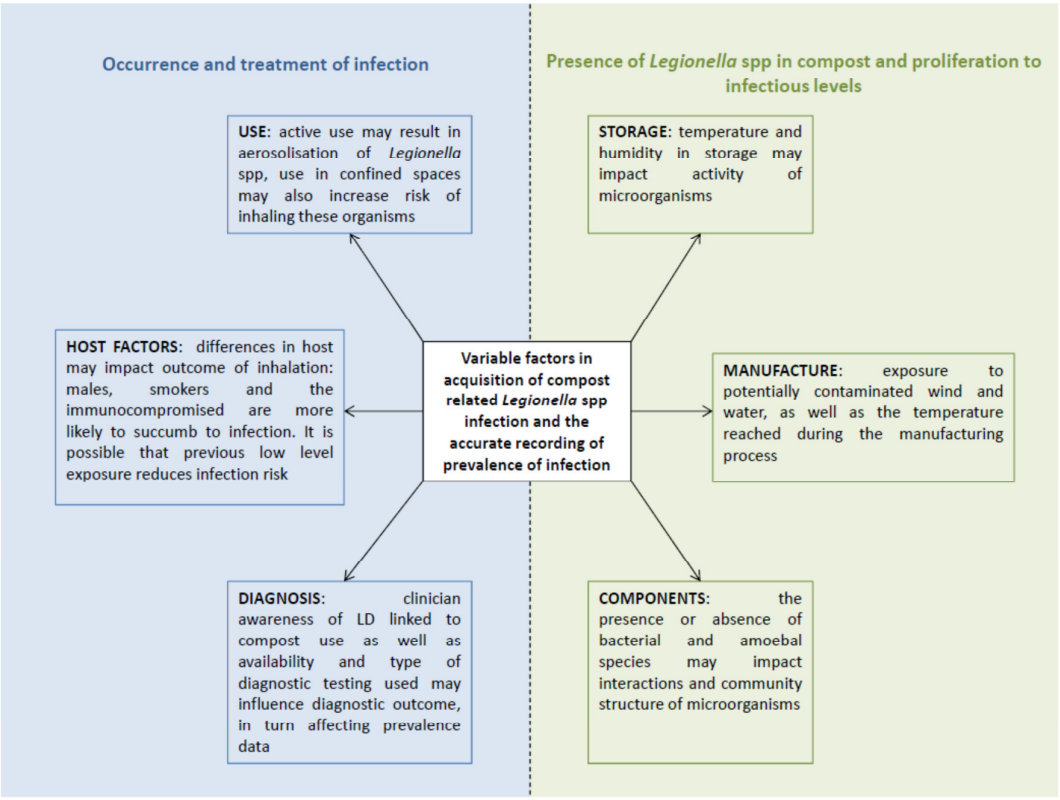
261 Figure 1 Variable factors related to the occurrence and recording of *Legionella* spp infections linked to

262 compost use



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